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Understanding biodiversity: The importance of sexual dimorphism in the micro- and macroevolution of hummingbirds

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**Understanding biodiversity: The importance of sexual dimorphism in the micro-
and macroevolution of hummingbirds**

by

Chelsea M. Berns

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Ecology and Evolutionary Biology

Program of Study Committee:
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TABLE OF CONTENTS

	Page
LIST OF FIGURES	iv
LIST OF TABLES	vi
ACKNOWLEDGEMENTS	vii
ABSTRACT	x
CHAPTER 1. GENERAL INTRODUCTION	
Introduction	1
Dissertation organization	7
Literature cited	10
CHAPTER 2. THE EVOLUTION OF SEXUAL DIMORPHISM: UNDERSTANDING MECHANISMS OF SEXUAL SHAPE DIFFERENCES	
Abstract	14
Sexual Size Dimorphism	17
Sexual Shape Dimorphism	19
Conclusion	30
Acknowledgements	33
Literature cited	34
CHAPTER 3. BILL SHAPE AND SEXUAL SHAPE DIMORPHISM BETWEEN TWO SPECIES OF TEMPERATE HUMMINGBIRDS: BLACK-CHINNED HUMMINGBIRD (<i>ARCHILOCHUS ALEXANDRI</i>) AND RUBY-THROATED HUMMINGBIRD (<i>A. COLUBRIS</i>)	
Abstract	41
Introduction	42
Materials and methods	47
Results	52
Discussion	56
Acknowledgements	61
Literature Cited	62
Appendix	69

CHAPTER 4. BECOMING DIFFERENT BUT STAYING ALIKE: PATTERNS OF SEXUAL SIZE AND SHAPE DIMORPHISM IN HUMMINGBIRDS

Abstract	72
Introduction	73
Materials and methods	78
Results	83
Discussion	89
Acknowledgements	95
Literature cited	96
Appendix A	100
Appendix B	102
Appendix C	112

CHAPTER 5. MACROEVOLUTIONARY PATTERNS OF SEXUAL SIZE AND SHAPE DIMORPHISM IN HUMMINGBIRDS

Abstract	118
Introduction	119
Materials and methods	125
Results	132
Discussion	140
Acknowledgements	144
Literature cited	146
Appendix A	153
Appendix B	160

CHAPTER 6. GENERAL CONCLUSIONS

Introduction	167
Chapter summaries	167
Literature cited	175

CURRICULUM VITAE	177
------------------------	-----

LIST OF FIGURES

Chapter 2

Figure 1. Differences between the sexes in coloration are common in many species, including the Eclectus parrot.....	14
Figure 2. Sexual size dimorphism in the poison dart frog	17
Figure 3. Rensch's Rule, where in species above the broken line (broken line denoting where female and male sizes are equal) females are larger than males and below, males are larger than females.....	18
Figure 4. Sets of linear distances	20
Figure 5. Maximum height and width taken on two different shapes results in the same linear measurement on both.....	21
Figure 6. Example of biologically homologous landmarks	22
Figure 7. Sexual shape dimorphism in eye stalks of <i>Teleopsis dalmanni</i>	29

Chapter 3

Figure 1. Curvature indices and landmark-based morphometrics, shown on a Black-chinned Hummingbird.....	49
Figure 2. Results of principal component analysis of all specimens (relative warp scores). Thin-plate spline deformation grids are shown to visualize patterns of shape variation	54
Figure 3. Thin-plate spline deformation grids and magnitude of sexual shape dimorphism	55

Chapter 4

Figure 1. a) Exposed culmen representing bill length. b) Landmark-based geometric morphometrics. c) Representative individual from the species with extreme bill curvature (<i>Calothorax lucifer</i>), and d) from the species with the straight bills (<i>Archilochus colubris</i>).....	79
Figure 2. Histograms displaying the variation of sexual size dimorphism within each clade of hummingbirds	87

Figure 3. Principal components plot of bill shape for the three species displaying significant sexual shape dimorphism (*A. colubris*, *S. scintilla* and *M. minima*). Lines connecting symbols represent the magnitude of sexual shape dimorphism. Thin-plate spline deformation grids of the average female and male bill shape relative to the mean are also presented 90

Figure 4. Current phylogeny of *Trochilidae* with emphasis on the *Mellisugini* clade. Representation of magnitude of sexual size dimorphism (SSD) and sexual shape dimorphism (SShD) 94

Chapter 5

Figure 1. Current phylogeny backbone of *Trochilidae*125

Figure 2. Exposed culmen (a), and landmark-based morphometrics, shown on a Black-chinned Hummingbird.....126

Figure 3. Subclades as determined by McGuire et al., 2007, 2009130

Figure 4. Sexual size dimorphism showing the clades that overlap in confidence intervals (a) and (b) sexual shape dimorphism showing the clades that overlap in confidence intervals134

Figure 5. Positive correlations between rate of evolution of sexual shape dimorphism, species richness and rate of species diversification.....139

Chapter 6

Figure 1. Diversity in *Trochilidae*.....169

LIST OF TABLES

Chapter 3

Table 1. Statistical results from linear measurements of maxillary curvature index, mandibular index, and the circle-curvature method	52
--	----

Table 2. Statistical results from landmark-based geometric morphometrics quantifying (A) between-species morphological variation and (B, C) within-species morphological variation	53
---	----

Chapter 4

Table 1. A) Statistical results from ANOVA on culmen length for the <i>Mellisugini</i> lineage. B) ANOVA comparing male versus female culmen lengths <i>Mellisugini</i> hummingbirds. Size dimorphism ratios for each species (expressed as Lovich-Gibbons ratios) are include	84
---	----

Table 2. A) MANOVA analysis of bill the <i>Mellisugini</i> clade. B) Results of permutational-MANOVA for those species displaying significant sexual shape dimorphism (<i>A. colubris</i> , <i>S. scintilla</i> and <i>M. minima</i>)	88
--	----

Chapter 5

Table 1. A) Rates of sexual size (SSD) and shape (SShD) dimorphism in each clade and B) 95% confidence intervals estimated from standard errors of each rate. Bold denotes those clades that overlap	135
---	-----

Table 2. Comparison between SSD and SShD rates of evolution, magnitude of sexual dimorphism and percent species with sexual dimorphism in each clade. Abbreviations: M_{SSD}/M_{SShD} = mean magnitude of SSD/SShD, $\%N_{SSD}/\%N_{SShD}$ = percent of species with SSD/SShD in each clade	136
--	-----

Table 3. Results of PGLS hypothesis testing of sexual size and shape dimorphism with multiple factor	137
---	-----

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ABSTRACT

For centuries, biologists have asked "why are there so many species, and why do we see such magnificent biological diversity?" This dissertation addresses this question by examining sexual size and shape dimorphism hummingbird bills at both a micro and macroevolutionary scale. Specifically, I quantify sexual size and shape dimorphism of bill morphology of 269 species of hummingbirds and begin with a species-level study to find that community structure might be a driving factor in the evolution of sexual size dimorphism in 2 sister species of hummingbirds, and the presence of sexual shape dimorphism in one of those species.

I then broaden my study to a clade-level analysis, examining the *Mellisugini* clade. I find that, while the majority of hummingbird species in the clade exhibit sexual size dimorphism, only 3 of 32 display shape dimorphism. Exploration of factors that may be underlying these patterns may be community structure, where species-poor communities tend to have a greater degree of sexual dimorphism than those in species-rich communities.

I scale up once more in a final study at the family level to investigate patterns of size and shape dimorphism in bill morphology. I find that there was no significant correlation between the mean magnitude of size dimorphism and the magnitude of shape dimorphism and that the rates of evolution of sexual size dimorphism did not predict those of shape dimorphism. This suggests that these traits are evolutionarily decoupled. I also found that the rate of sexual shape dimorphism, species richness, and rate of

species diversification are all positively correlated, implying that these are predictors of species richness. These relationships are consistent with the macroevolutionary theory of ‘punctuated equilibrium’, but also may result from the opening of new niches and the presence of ‘adaptive zones’. In combination, I demonstrate that microevolution alone cannot explain macroevolution, but requires the integration of processes at multiple hierarchical scales and in this dissertation, I elucidate a rich picture of the evolution of diversity.

CHAPTER 1. GENERAL INTRODUCTION

Overview

For centuries, biologists have asked "why are there so many species, and why do we see such magnificent biological diversity?" Natural philosophers such as Aristotle and Linnaeus attempted to organize and understand observable patterns of biological diversity, but it was not until Darwin that an explanatory evolutionary theory came into focus. Darwin's two key contributions of natural selection and descent with modification provided both a natural mechanism for evolutionary change and a description of the resulting patterns through time (Darwin, 1859). Armed with this evolutionary theory, biologists can now examine the underlying mechanisms of phenotypic accumulation and the patterns that result, ultimately addressing how biological diversity is generated and maintained.

One important component of biodiversity that biologists wish to study is phenotypic diversity, which is witnessed by the fascinating organismal diversity that we observe. In order to understand how this biodiversity arises, one approach is to examine phenotypic diversity from a macroevolutionary (above the species level) perspective. However, to understand the processes that lead to this grand scale of evolution at a macro-perspective, the examinations of microevolutionary (below the species level) processes are essential (Futuyama, 2005). Thus, for a richer description of the patterns and mechanisms influencing biological diversity, it is necessary to understand the patterns

and processes at multiple hierarchical scales; first examining what occurs within species (micro-evolution), and, once these processes are explored, one identifying the processes underlying evolution above the species level (macroevolution).

Understanding patterns and processes at the macroevolutionary level requires knowledge of the interplay between the ecology of organisms, selection at contemporary timescales, and the phylogenetic history among species. Armed with this paradigm, biologists can understand how diversification occurs among lineages, and may evaluate alternative models of phenotypic evolution and change. In this dissertation I use this approach to pursue one broad objective via several integrated and hierarchical studies. Using hummingbirds as a model system, I examine the patterns and underlying processes of phenotypic diversity at both micro- and macroevolutionary scales by starting with a pair of sister species, scaling up to their tribe, and finally to the entire family to quantify how the tempo of evolution, clade age, diversification rate, and species richness impact hummingbird diversity.

Tempo and Mode in Macroevolution

Understanding how taxonomic and phenotypic diversity accumulate over time has been a longstanding goal of evolutionary biologists. Darwin was interested in the rate (tempo) of evolution and posited that small variations accumulate over time until a new species is born (Darwin, 1859), however it was not until Simpson (1944, 1953) that rates of evolution were quantified and three main causal mechanisms were proposed. One is speciation, when similar species mix within a single adaptive zone and the appearance of

only minor variation occurs in the same kind of species. Here, it may be expected that evolutionary rates would be erratic and there would be no general trend. A second mechanism is termed the 'phyletic' mode, whereby all species in a lineage are changed by sustained selection, predicting a moderate rate of change. The third is 'quantum evolution,' which predicts drastic shifts in adaptive zones and rapid rates of evolutionary change (Simpson, 1944, 1953).

With the knowledge that these mechanisms can affect evolutionary rates, one can expect that when lineages first enter "adaptive zones," (a set of ecological niches occupied by a group of species exploiting the same resources in a similar manner: Simpson 1944) morphological evolution should be rapid as these zones become filled. Under this hypothesis, rates of morphological evolution should then slow after species initially exploit new niches (Foote, 1994; Schluter, 2000; Simpson, 1944; now termed "Early Burst" models of evolution). However, an alternative model predicts that over evolutionary time at the macroevolutionary level, there may be long periods with little change (stasis) that are interrupted by periods of higher rates of change (punctuation events) (termed "punctuated equilibrium", Gould and Eldredge, 1977). When this is the case, one can predict that species which generally exhibit stasis will have rates of morphological change that are positively correlated with rates of speciation. Major evolutionary theories such as these present many testable predictions.

For instance, one might expect that there is a relationship between species diversity and morphological disparity if adaptive evolution continues over long time spans. This pattern can be the result of some selective (adaptive) process, such as change

in gene pool composition or community structure, occurring gradually over time (Ricklefs, 2004, 2006a). It can also be predicted that rates of morphological evolution increase when rates of species diversification are elevated, perhaps because lineages with these high rates diversify more than those with low rates of morphological evolution (Harmon et al., 2003; Mahler et al., 2010). Some authors predicted that increased rates of morphological evolution may be due to the evolution of phenotypic adaptation facilitated by the ability of a clade to exploit an ecological space (Martin and Wainwright, 2011; Schluter, 2000). If this prediction holds, we might expect that higher rates of phenotypic evolution correspond to the ability to move into new niches and that these clades will have greater diversification and species richness as compared to those clades that cannot. Conversely, a decrease in rates of speciation and lower species diversity may impact the independence of age and species richness (Purvis, 2004; Ricklefs, 2007; Ricklefs, 2006b). My dissertation elucidates patterns of the accumulation and maintenance of diversity by addressing evolutionary rates, sexual dimorphism, clade age, species richness and rates of species diversity to provide a rich description of the evolution of morphological diversity.

Sexual Dimorphism

Studies such as those described above generally focus on the evolutionary tempo and mode of single, univariate traits, such as body size (Harmon et al., 2010), proportion of limb length (Mahler et al., 2010), and aspects of body shape to quantify rates of morphological evolution. In such studies, each species is represented by a single value per trait (typically the mean), which is used in a phylogenetic framework to obtain

estimates of the rate of evolution of that trait. However, evolutionary biologists are also interested in how other species-level characteristics evolve, such as sexual dimorphism (SD). Sexual dimorphism is a trait wherein males and females of the same species differ phenotypically, and it is pervasive throughout the animal kingdom. The consequences of SD on the ecology and evolution of organisms are often profound, and thus, it is a crucial trait to examine in order to further understand the processes of diversity (Berns, 2013; Berns and Adams, 2013; Butler et al., 2007; Fairbairn et al., 2007).

Sexual dimorphism can be exhibited in a myriad of traits, such as differences in coloration (Endler, 1983), behavior (Clyne and Miesenböck, 2008), vocalizations (Price, 1998), ornamentation (Worthington et al., 2012), or body size (sexual size dimorphism; Berns and Adams, 2010; Stephens and Wiens, 2009). A variety of mechanisms have been proposed to explain patterns of SD. The primary one is sexual selection (Abouheif and Fairbairn, 1997; Andersson, 1994; Hedrick and Temeles, 1989), a form of natural selection that enhances fitness in relation to reproduction (Darwin, 1871; Jones and Ratterman, 2009). Sexual selection can drive each sex to develop different adaptations that enhance their possibilities of transmitting their genes onto the next generation, impacting patterns of phenotypic diversity (see “Chapter 2” for a full description of mechanisms underlying the evolution of SD). While mechanisms underlying the evolution of SD continue to be examined, curiously, rates of evolution of sexual dimorphism have received considerably less attention. In my dissertation, I investigate rates of sexual size and shape dimorphism in hummingbirds in an effort to understand how patterns of sexual dimorphism evolve at macroevolutionary scales.

Hummingbirds

Hummingbirds (family *Trochilidae*) are a diverse family with 338 currently recognized species (McGuire et al., 2007; McGuire et al., 2009; Birdlife.org, 2012). This family has been a model taxon for the study of sexual dimorphism (SD), as many species exhibit a myriad of patterns such as dimorphic plumage (Bleiweiss, 1992a, b, 1997; Darwin, 1871), sex-specific behavior (Feinsinger and Colwell, 1978; Kodric-Brown and Brown, 1978), ornamentation (Evans et al., 1995; Zusi and Gill, 2009), and even bill morphology (Berns, 2013; Berns and Adams, 2013; Temeles, 1996; Temeles and Kress, 2003; Temeles et al., 2000). In this group it has long been observed that bill morphology is under strong selection pressures due to foraging efficiency and this has been implicated as the major cause of trophic differences between species (Brown and Bowers, 1985; Montgomerie, 1984). Not only do species forage differently relative to one another, but research has also found that the sexes may forage differentially in particular species (Carpenter et al., 1991; Temeles and Kress, 2003). Indeed, many of these species display sexual dimorphism in their bill size and shape (Temeles et al., 2010). There is now quantitative support that there is a direct link between dimorphism in bill morphology and sex-specific foraging in *Eulampis jugularis* (Purple-throated Carib), such that males and females forage on morphologically different resources, and the bill morphology of the sexes is correlated with the morphology of the flower that each feeds from (Temeles et al., 2000). This has been observed in other species of hummingbirds as well (Temeles et al., 2010), which suggests that SD in bill morphology is an evolutionary result from

sex-specific ecological processes (for further detail see Chapters 3-5). The question though, is whether or not this relationship is found more broadly across hummingbird taxa, or whether it is specific to this species. In this dissertation, I use a phylogenetically hierarchical approach to examine microevolutionary patterns of sexual dimorphism in bill size and shape across taxa in an effort to determine the prevalence of such patterns at the macroevolutionary level, and infer the processes that may have caused them.

Dissertation Outline

In Chapter 2 (published as Berns, 2013) I begin by reviewing, outlining and describing several hypotheses and mechanisms that explain patterns of sexual dimorphism within and among hummingbird taxa. I provide a literature review of studies that focus on what is known about biological patterns and underlying processes specifically regarding sexual shape dimorphism, the methods used to quantify this and the patterns that arise from these studies. Finally, I explore why differences in the degree of sexual size dimorphism may be observed.

In Chapter 3 (published as Berns and Adams, 2010) I quantify sexual size and shape dimorphism in the bills of two sister species of hummingbirds: *Archilochus alexandri* (Black-chinned Hummingbird) and *Archilochus colubris* (Ruby-throated Hummingbird) from the *Mellisugini* hummingbird clade. In order to quantify bill shape, I not only use geometric morphometric methods, but also employ three commonly used linear measurement methods, as described above, to compare these three approaches and quantify shape. In comparing patterns of size and shape dimorphism between these two

species, I address the questions: 1) Do closely related species display differing degrees of sexual size and shape dimorphism? and 2) How do community structure, competition, and life history traits such as breeding affect patterns of shape and size dimorphism? In doing so, I shed light into the ecological factors that affect patterns of microevolution.

In Chapter 4 (in press as Berns and Adams, 2013) I apply a clade level analysis to examine patterns of phenotypic evolution in bill morphology of 32 of the 35 species in the *Mellisugini* hummingbird clade. In addition to using geometric morphometric methods to calculate bill shape, I combine linear measurements taken from my data and those from the literature of 219 hummingbird species to quantify presence and magnitude of sexual size dimorphism. In taking a clade-level perspective, I ask: 1) Do more closely related species share more similar patterns of sexual size and shape dimorphism? 2) What are the influential ecological mechanisms underlying patterns of size and shape dimorphism? and 3) What are the evolutionary trends of dimorphism both within one clade, and across *Trochilidae* in general? In examining both micro- and macroevolution, I provide a richer understanding of how microevolutionary mechanisms impact macroevolutionary patterns.

In Chapter 5 (Berns and Adams, 2013, in prep.) I examine patterns of phenotypic evolution at a macroevolutionary scale, using bill morphology of 269 hummingbird species to address the following evolutionary questions: 1) What are the patterns of sexual size and shape dimorphism in hummingbird bill morphology? 2) Are sexual size and shape dimorphism correlated such that the rates of evolution of size and shape dimorphism significantly influence one another? 3) What impacts patterns of phenotypic

accumulation: rates of evolution of sexual dimorphism, clade age, percent of species with bill dimorphism, species richness, magnitude of dimorphism, and/or species diversification? In addressing these macroevolutionary questions, I provide unique insight into the diversity of hummingbirds and the patterns of diversity that they exhibit.

Finally, in Chapter 6 I describe the hypotheses of each chapter and my results. I then provide context to further explore the implications of my findings in both a micro- and macroevolutionary context.

Conclusion

Understanding the mechanisms and patterns of diversity has presented a long-standing challenge to evolutionary biologists. Studies continue to examine the microevolutionary patterns and processes that influence macroevolutionary biodiversity. The results of this dissertation show that at microevolutionary scales, ecology clearly influences patterns of dimorphism, however at a higher levels, shared evolutionary history has profound effects on hummingbird biodiversity as well. This highlights the importance of coupling research across a hierarchy of phylogenetic scales to provide insights at numerous levels of biological organization. In so doing, my dissertation more fully addresses the question "why are there so many species, and why do we see such magnificent biological diversity?"

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CHAPTER 2

THE EVOLUTION OF SEXUAL DIMORPHISM: UNDERSTANDING MECHANISMS OF SEXUAL SHAPE DIFFERENCES

A book chapter accepted for publication in “Sexual Dimorphism”, edited by Professor

Hiroshi Moriyama (ISBN 978-953-51-1075-0)

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Introduction

Understanding the origin of biodiversity has been a major focus in evolutionary and ecological biology for well over a century and several patterns and mechanisms have been proposed to explain this diversity. Particularly intriguing is the pattern of sexual dimorphism, in which males and females of the same species differ in some trait. Sexual dimorphism (SD) is a pattern that is seen throughout the animal kingdom and is exhibited in a myriad of ways. For example, differences between the sexes in coloration are common in many organisms (Stuart–Fox and Ord 2004) ranging from poeciliid fishes (Endler 1983) to dragon flies (Moore) to eclectus parrots (see Figure 1).



Figure 1. Differences between the sexes in coloration are common in many species, including the Eclectus parrot.

Sexual dimorphism is also exhibited in ornamentation, such as the horns of dung beetles (Watson and Simmons 2010), the antlers of cervids (Geist and Bayer 2009), and the tail of peacocks (Loyau et al. 2005). Many species also exhibit sexual differences in foraging behavior such as the Russian agamid lizard (Ananjeva and Tsellarius 1986), and parental behavior and territoriality can be dimorphic in species such as hummingbirds (Stiles 1971, Armstrong 1987). Another common pattern is that of sexual size dimorphism, such as is observed in snakes (Shine 1978) and monk seals (Ralls).

There are many mechanisms that drive the evolution of SD, the most accepted mechanism being sexual selection (Hedrick and Temeles 1989, Andersson 1994, Abouheif and Fairbairn 1997), which enhances fitness of each sex exclusively in relation to reproduction (Darwin 1871, Jones and Ratterman 2009a). This states that SD evolves in a direction such that each sex (especially males, see Stuart-Fox 2009) maximizes reproductive success in two ways: by becoming more attractive to the other sex (inter-sexual dimorphism) or by enhancing the ability to defeat same-sex rivals (intra-sexual dimorphism), in both cases such that each sex increases the chances to mate and pass genes on to the next generation. Many researchers have argued that competition for mates is at the very heart of sexual selection because these rivalries greatly influence mating and fertilization success. Indeed, competition for mates has been shown to be the major factor impacting SD in several taxa (Bean and Cook 2001). However the complexity of SD cannot be explained by a single mechanism.

Mate choice is an important proximate mechanism of sexual selection. Often the sex with the higher reproductive investment is the 'choosy' sex. Patterns then emerge,

such as those consistent with the ‘sexy son’ hypothesis (Weatherhead and Robertson 1979), where females prefer mates with phenotypes signifying fitness. The females prefer males that are phenotypically ‘sexy’ to ensure that the genes of their offspring will produce males that will have the most breeding success, propagating her genes successfully (Hunt et al., Jones and Ratterman 2009a). Taken further, sometimes females prefer males that exhibit very extreme phenotypes within a population. Over evolutionary time these traits become increasingly exaggerated despite the potential fitness costs to the males themselves, termed Fisherian runaway sexual selection (Weatherhead and Robertson 1979). Examples include the tails of male peacocks, plumage in birds of paradise and male insect genitalia (Fisher 1915, 1930, Andersson 1994).

Alternatively, ecological mechanisms, such as competition for resources, may exert distinct selective forces on the sexes, resulting in the evolution of SD (Eberhard 1985). Here, intraspecific competition in species-poor communities may allow divergent selection between the sexes (rather than between species), resulting in sexual niche segregation (Selander 1972, Slatkin 1984, Hedrick and Temeles 1989, Shine 1989). In this case morphological traits often change to minimize this intersexual competition. Other ecological hypotheses have been proposed to explain patterns of SD, such as the influence of sex-specific divergence in response to environmental gradients (i.e., intersexual niche packing: *sensu* Butler et al. 2000). For example, both sexes of fruit flies *Drosophila subobscura* increase in body size with latitude, however in South America these size increases are less steep and weaker in males as compared to females

(Hendry et al. 2006b). Another study found weaker latitudinal clines in males as compared to females in houseflies *Musca domestica* (Lovich and Gibbons 1992), and yet another study found geographical variation in climate that corresponded to a change in the magnitude of sexual size dimorphism between males and females (Stephens and Wiens 2009). Hypotheses continue to be proposed and the explanations for the evolution of SD may not be mutually exclusive but instead, may operate in a synergistic or antagonist fashion to shape these patterns.

Processes and Patterns of Sexual Size Dimorphism

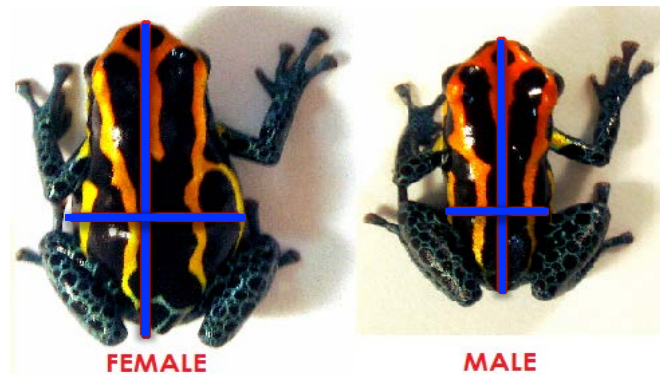


Figure 2: Sexual size dimorphism in poison dart frog.

Sexual size dimorphism is a frequent phenomenon where the size of males and females of the same species differ (see Figure 2), driven by one or more of the mechanisms mentioned above. When these processes occur in closely related species, distinct patterns of among-species size dimorphism can result, one of which is termed ‘Rensch’s Rule’ (Rensch 1950). Rensch’s rule is a pattern wherein the degree of sexual size dimorphism

increases with body size in species where males are the larger sex, and conversely decreases in those species where females are the larger sex (see Figure 3).

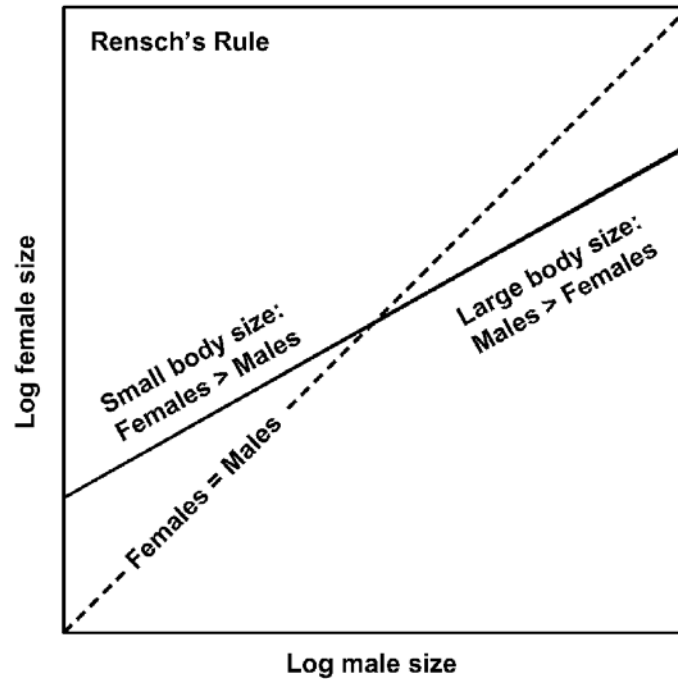


Figure 3. Rensch's Rule, where in species above the broken line (broken line denoting where female and male sizes are equal) females are larger than males and below, males are larger than females. From R. Colwell, *Am. Nat.*, 2000.

Several hypotheses have been proposed to explain Rensch's rule. One proposes that the combination of genetic correlations between male and female size with directional sexual selection for larger male size will cause the evolution of larger males relative to female body size (Fairbairn and Preziosi 1994, Abouheif and Fairbairn 1997, Fairbairn 1997). Another argues that sexual size dimorphism evolves through intraspecific competition between the sexes when foraging is related to size (Darwin 1871, Shine 1989). Finally, many researchers have hypothesized that this pattern is due

to female fecundity, where the larger female will have bigger eggs and a greater capacity to reproduce successfully (Darwin 1871, Williams 1966, Hughes and Hughes 1986). Examples of Rensch's rule and support for all three hypotheses abound in nature in organisms as diverse as hummingbirds (Colwell 2000), hummingbird flower mites (Colwell 2000), water striders (Fairbairn and Preziosi 1994), turtles (Berry and Shine 1980), salmon (Young 2005) and shorebirds (Székely et al. 2004).

Another such pattern is that of 'adaptive canalization', where the larger sex has less plasticity compared to the smaller sex. This is due to directional selection for a large body size and individuals with sub-optimal body sizes will have lower fitness (Fairbairn 2005, Blanckenhorn et al. 2006). Alternatively, there may be condition-dependence, where the larger sex is under stronger directional selection for a large size and will be more affected by different environmental factors as compared to the smaller sex. This indicates that sexual size dimorphism should change with changing environments. These hypotheses and studies have led to much understanding of the patterns and processes underlying sexual size dimorphism.

Sexual Shape Dimorphism

In addition to sexual size dimorphism, males and females often differ widely in shape (Hendry et al. 2006a, Butler et al. 2007). Curiously, although shape can contribute meaningfully to various functions such as feeding, mating, parental care, and other life history characteristics, patterns of sexual shape dimorphism have historically received considerably less attention than sexual size differences (Lande and Arnold 1985, Hedrick

and Temeles 1989, Gidaszewski et al. 2009). Examining the size and shape of traits together provides a much more complete quantification of sexual dimorphism, as the two components are necessarily related to one another. As such, shape analysis allows a deeper understanding of mechanisms underlying SD, because different parts of the body can serve multiple functions and be under distinct selective regimes.

Shape is defined as the specific form of a distinct object that is invariant to changes in position, rotation and scale (Bookstein 1991, Dryden and Mardia 1998), and many methods have been proposed to study shape. For instance, sets of linear distances may be measured on each individual (e.g., length, width and height) to represent shape (Figure 4A), as well as angles (Figure 4B) and ratios of these measurements.

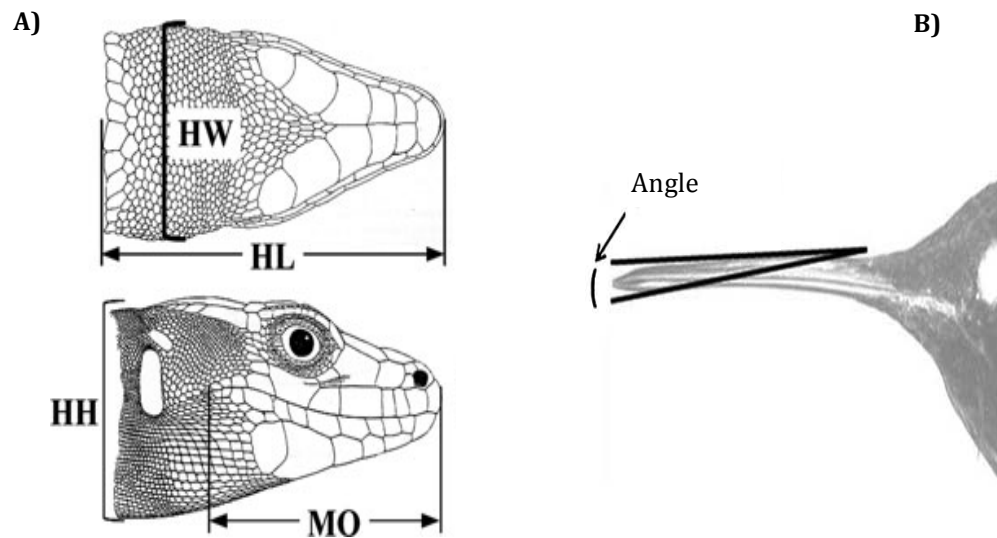


Figure 4. A) Sets of linear distances: Head length (HL), head width (HW), head height (HH), and mouth opening (MO) and B) Measurement of angle. A): adapted from Kaliontzopoulou et al. 2012. B): adapted from Berns and Adams, 2010.

Sets of linear distances do not always accurately capture shape because of shortcomings that limit their general utility. For instance, it is possible that for some

objects the same set of distance measurements may be obtained from two different shapes, because the location of the measurements is not recorded in the distance measures themselves. For example, if the maximum length and width were taken on an oval and teardrop, the linear values might be the same even though the shapes are clearly different (see Figure 5).

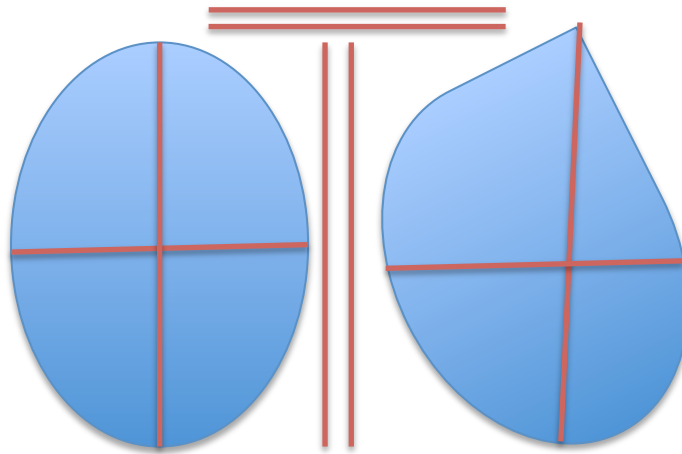


Figure 5. Maximum height and width taken on two different shapes results in the same linear measurement on both.

Additionally, it is not possible to generate graphical representations of shape using these measurements alone because the geometric distances among variables is not preserved and aspects of shape are lost (Adams et al. 2004). As a result of these shortcomings, other analytical approaches for quantifying shape have been developed.

A major advance in the study of shape is landmark-based geometric morphometric methods, which do not have these difficulties. These methods quantify the shape of anatomical objects using the Cartesian coordinates of biologically homologous landmarks whose location is identified on each specimen (Figure 6). These landmarks

can be digitized in either two- or three-dimensions, and provide a means of shape quantification that enables graphical representations of shape (see Figure 6).

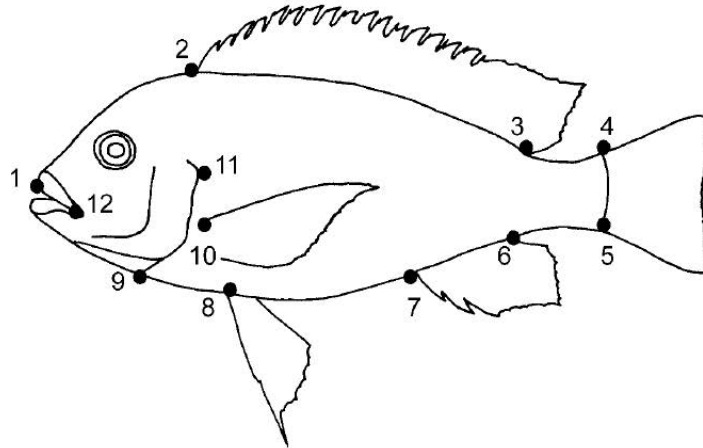


Figure 6. Example of biologically homologous landmarks. From Kassam *et al.* 2003.

Geometric morphometric analyses of shape are accomplished in several sequential steps. First, the landmark coordinates are digitized from each specimen. Next, differences in specimen position, orientation and size are eliminated through a generalized Procrustes analysis. This procedure translates all specimens to the origin, scales them to unit centroid size, and optimally rotates them to minimize the total sums-of-squares deviations of the landmark coordinates from all specimens to the average configuration. The resulting aligned Procrustes shape coordinates describe the location of each specimen in a curved space related to Kendall's shape space (Bookstein et al. 1999, Slice 2005). These are then projected orthogonally onto a linear tangent space yielding Kendall's tangent space coordinates (Dryden and Mardia 1993, Dryden and Mardia 1998, Rohlf 1999), which can then be treated as a set of shape variables for further analyses of

shape variation and covariation with other variables (e.g., Adams et al. 2007, Adams 2010, Adams and Nistri 2010).

In terms of sexual shape dimorphism, sets of both linear measurements and geometric morphometric methods have been utilized to identify patterns of shape dimorphism in numerous taxa, including fish (Herler et al. 2012), turtles (Ceballos and Valenzuela 2011), birds (Temeles et al. 2005, Navarro et al. 2009, Berns and Adams 2010, Berns and Adams 2012) and lizards (Kuo et al. 2009, Kaliontzopoulou et al. 2010). In addition to quantifying sexual shape dimorphism, identifying the potential mechanisms that generate these patterns is a current focus of many evolutionary biologists. For instance, one central hypothesis for the evolution of sexual shape dimorphism is that males and females diverge phenotypically due to intersexual competition for similar resources. Here, functional morphological traits diverge between the sexes such that the sexes partition resources. Under this scenario, SD is more strongly influenced by natural selection than sexual selection. For example, in the cottonmouth *Agkistrodon piscivorous*, sex-specific prey consumption as a function of prey size is directly correlated with differences in head morphology between males and females (Vincent et al. 2004). Thus natural selection, and not sexual selection, maintains both foraging and morphological differences between the sexes in this species.

By contrast, sexual shape dimorphism can be the result of sexual selection. For example, in the tuatara *Sphenodon punctatus*, Herrel et al. (2009) tested the hypothesis that sexual shape dimorphism is due to niche differentiation between the sexes, rather than driven by the territoriality of males. Head shape is much larger in males as

compared to females and this may be functionally tied to the larger prey of males. The authors suggested that sexual selection for male-male combat may play a role, but that bite force differences between males and females may be impacting the maintenance of these sexual differences. Interestingly, it was found that males do have a greater bite force relative to females, but that these differences and their maintenance are the result of sexual selection, as bite force is correlated with good male condition but not with female condition (Herrel et al. 2010).

Another study also rejects the hypothesis that differential niches maintain sexual shape dimorphism. Feeding, territory, and mate acquisition have been proposed as functions for the bill of the Cory shearwater *Calonectris diomedea* (Navarro et al. 2009). The bill morphology is such that sexual differences are related not to feeding ecology, but to sexual selection and antagonistic interactions. On the other hand, the Purple-throated Carib (*Eulampis jugularis*) hummingbird exhibits the clear link between function and the different food preference of males and females, suggesting that the longer and more curved bill of the female as compared to the male is due to the division of resources (Temeles et al. 2000, Temeles and Kress 2003, Temeles et al. 2010). In other species of hummingbirds that exhibit sexual size and shape dimorphism in their bills however, it is unclear whether interspecific competition and niche differentiation, sexual selection, or some other force drives this sex-specific morphology (Berns and Adams 2010, Berns and Adams 2012).

One study investigated the relative contributions of intersexual resource partitioning and sexual selection in the amagid lizard *Japalura swinhonis* (Kuo et al.

2009). Here, sexual shape dimorphism was not correlated with diet, however limb size and shape were associated with perch habitats. These findings are inconsistent with the hypothesis of intraspecific competition for resources, but provide evidence for the ‘fecundity advantage’ hypothesis. Under this hypothesis, a large mother can produce more offspring than a small mother, and can give her offspring better conditions through directional selection (Andersson 1994). For instance, an increase in abdominal volume can arise with an increase in overall body size, seen in some mammals and amphibians (Monnet and Cherry 2002, Tague 2005), or in the abdomen’s relative proportion to overall body size, like that of some reptiles (Schwarzkopf 2005). Olsson et al. (2002) examined SD in the heads and trunk length of an Australian lizard *Niveoscincus microlepidotus* to address the hypothesis that head morphology dimorphism had evolved via sexual selection for male-male combat and that trunk length evolved due to fecundity selection. Results did not uphold one part of this prediction however, as sex divergence in head morphology was genetic and not specifically due to sexual selection. Evidence was presented in favor of the prediction that difference in trunk length is driven by fecundity advantage, and that sexual selection favored males with smaller trunk size. Studies such as these suggest that sexually dimorphic shape traits may be driven by the combination of natural selection for fecundity advantage and by sexual selection.

Evidence supporting fecundity advantage is weak or not existent in many systems however. For instance, investigators examining the tortoise *Testudo horsfieldii* hypothesized that the wider shells of the females provided more room for eggs, but were unable to provide conclusive evidence for fecundity advantage. Instead, the patterns of

sexual shape dimorphism seemed to be due primarily to locomotive constraints of mate seeking and male-male combat (Bonnet et al. 2001). In two species of crested newt *Triturus cristatus* and *T. vulgaris*, results somewhat support fecundity advantage, however researchers suggest there are more underlying processes driving the evolution of sexual shape dimorphism than simply fecundity selection (Malmgren and Thollessen 1999). Evidence presented by Willemsen and Haile (2003) outright reject the fecundity advantage hypothesis. Three tortoise species *Testudo graeca*, *T. hermanni*, and *T. marginata* have differing courtship behaviors and display differing magnitudes of sexual shape dimorphism dependent on their specific courtship display. In contrast to previous studies, the authors suggest that these results indicate that sexual shape dimorphism is driven not by fecundity advantage and natural selection, but rather by sexual selection. From the inconcordant results of studies such as these, it remains unknown whether patterns of the evolution of sexual shape dimorphism are primarily driven by natural selection for fecundity advantage or by some other mechanism.

Environmental conditions are also hypothesized to drive the evolution of different shapes between the sexes. Evidence for one environmentally-driven hypothesis is presented in a study looking at environmental gradients underlying SD and parallel evolution of a species of guppy *Poecilia reticulata* (Hendry et al. 2006b). Results indicate that populations experiencing high predation were made up of males with smaller heads and deeper caudal peduncles. Open canopy sites resulted in selection for females with smaller heads and distended abdomens, whereas both sexes in high flow sites had small heads and deeper caudal peduncles. Males and females showed some shared responses to

the environmental gradients, thus indicating that environmental variables may be responsible for sexual shape dimorphism more than sexual selection pressures might be. More support for the hypothesis that environmental processes drive variation in sexual shape dimorphism is found in the Greater Antillean *Anolis* lizards that exhibit sexual size and shape dimorphism. Males and females use habitats differently and although sexual size dimorphism is not highly related to habitat use, sexual shape dimorphism is (Butler and Losos 2002). Further study on West Indian *Anolis* lizards also suggests environment as a major factor driving the patterns of sexual shape dimorphism. Concordant with the Greater Antillean *Anolis* lizards, the shape dimorphism clearly reflects the different niches occupied by males and females (Butler et al. 2007).

Although these and numerous other examples demonstrate the influence of environment on the evolution of sexual shape dimorphism, a recent study examined sexual shape dimorphism in the snapping turtle *Chelydra serpentina* and found no evidence that environmental condition was correlated with shape dimorphism. Unlike sexual size dimorphism, shape dimorphism was evident at turtle hatching and at 15.5 months, where both males and females exhibited this pattern under optimal and suboptimal conditions. When adults however, sexual size dimorphism was present and differed under conditions such that there is increased plasticity of the larger sex as compared to the smaller. Interestingly however, sexual shape dimorphism still did not vary with differing conditions (Ceballos and Valenzuela 2011). It has been suggested for over a century that environment is a major driver of morphological differences (Darwin

1859, Grant and Grant 2002), and new evidence such as this presents an opportunity to further understand the variables at play in the evolution of shape dimorphism.

Broadly, allometry (defined as a change in shape related to a change in size: Gidaszewski et al. 2009) has also been suggested as having an influential impact on sexual shape dimorphism (O'Higgins and Collard 2002, Schaefer et al. 2004). In an example of evolutionary allometry, Gidaszewski et al. (2009) examined sexual shape dimorphism in the wings of nine species of *Drosophila melanogaster* in a phylogenetic framework. Sexual shape dimorphism diverged among the nine species, however the evolution of sexual shape dimorphism was constrained by evolutionary history. This provides evidence that, while allometry is a large part of the evolution of sexual shape dimorphism in this system, it is not the main factor driving shape dimorphism. Kaliontzopoulou et al. (2008) studied heterochronic patterns of allometry in two species of lizard, *Podarcis bocagei* and *P. carbonelli*. Here, allometry did influence sexual shape dimorphism such that males and females actually differed in allometry with respect to head shape and body size, where change in male size increased disproportionately relative to head size and dimensions. Yet another recent study on sexual shape dimorphism in the stalk-eyed fly *Teleopsis dalmanni* found conclusive evidence for the impact of allometry on sexual shape dimorphism, where the size of the eye bulbs decreased with an increasing eye span and eyestalks became more elongated as they became thinner (Figure 7; Worthington et al. 2012).



Figure 7. Sexual shape dimorphism in eye stalks of *Teleopsis dalmanni* (Photo credit: Jerry Husak).

Exceptions continue to be found however. For instance, in a recent study examining sexual size and shape dimorphism in the bill morphology of two hummingbirds *Archilochus colubris* and *A. alexandri*, Berns and Adams (2010) examined a model that included an allometric component. This model was found to be inferior to those that included size, shape, and sex, and when graphically depicted, it was clear that allometry is a less influential factor in the evolution of sexual shape dimorphism. However, as demonstrated by the other studies described above, it seems that allometry is generally an important process driving the patterns of evolution in sexual shape dimorphism.

Conserved genetics may be yet another factor driving patterns of sexual shape dimorphism. Sexual shape dimorphism has been studied in the piophilid fly *Prochyliza*

xanthostoma (Piophilidae) and *Telostylinus angusticollis* (Neriidae) to address the impact of conserved genetic factors on patterns of sexual shape dimorphism (Bonduriansky 2006). These related species share similar patterns of sexual shape dimorphism, but have drastically different ecological and functional requirements as well as male-female interactions. Given that shape dimorphism is the similar trait in these three species, these flies may have congruent patterns of shape variation interspecifically, not due to common life-history requirements (Bonduriansky 2006). Bonduriansky suggests that this may be due to conserved genetics common to either species, or a reflection of interspecific variation in selection. In 2007, Bonduriansky (Bonduriansky 2007) performed another study on *Telostylinus angusticollis* to examine condition-dependence and genetic variation. Sexual dimorphism is significantly correlated with the condition such that these two traits share a common genetic (and developmental) base. Therefore, it is possible that in this, as well as other systems, sexual shape dimorphism is a pleiotropic effect where sex-linked genes determine the allocation of traits differently in males and females. Any variation of these genes due to plasticity may then determine the genetic relationship of sexual shape dimorphism and differing conditions. Many genetic hypotheses continue to examine sexual size dimorphism and just recently is sexual shape dimorphism receiving attention.

Conclusion

Although studies are currently underway, many questions about sexual shape dimorphism still remain. For instance, how frequently is sexual shape dimorphism

exhibited and how is this related to ontogenetic and biomechanical influences?

Worthington et al. (2012) propose that the sexually dimorphic patterns seen in the stalk-eyed fly are due to sexual selection, but also to biomechanical and possibly ontogenetic constraints. However, more information is necessary before a conclusion can be made about the actual process underlying the pattern of sexual shape dimorphism.

Kaliontzopoulou et al. (2008) suggest that a lack of sexual differences in cranial development of *Podarcis* species may indicate an ontogenetic limitation on both sexes, but also note that the habitat appears free of head constraint. The examination of ontogenetic development as well as biomechanical constraints on sexual shape dimorphism may reveal much about the causes and selective forces of these patterns, many of which are still unknown.

Does sexual shape dimorphism follow well-known patterns of sexual size dimorphism, such as Rensch's Rule? How much impact does allometry have in driving the evolution of sexual shape dimorphism? Although patterns such as these have been suggested as a component of sexual shape dimorphism, only recently have researchers begun to investigate these patterns. Is allometry in sexual shape dimorphism common? Berns and Adams (2010) did not find a significant effect of allometry, whereas Worthington et al. did (2012). In species of *Drosophila melanogaster*, allometry did not explain the majority of evolutionary divergence of sexual shape dimorphism (Gidaszewski et al. 2009), while in *Podarcis bocagei* and *P. carbonelli*, Kaliontzopoulou et al. (2008) showed that allometry was present and even differed in males and females. These inconcordant results suggest that there is a multifaceted interaction between sexual

size dimorphism, sexual shape dimorphism and allometry. Examining size alone shows only a piece of the mechanisms contributing to allometry, thus attention needs to also focus on allometry and its relationship with sexual shape dimorphism.

As seen in the examples in this chapter, much of the evidence on processes underlying sexual shape dimorphism is incongruent. One area needing attention is that of the correlation between sexual shape dimorphism and fecundity advantage, as shape may impact egg carrying capacity as size does. More work is needed to assess genetics and sexual shape dimorphism, and studies continue to argue that sexual selection causes sexual shape dimorphism due to male-male combat and mate choice, while others argue for natural selection via environmental factors and interspecific competition. No doubt that all of these factors play a role in influencing the evolution of sexual shape dimorphism, but what are the patterns? Do vertebrates tend to follow one trend while invertebrates follow another? In closely related species, does body size impact the effect of condition dependent sexual shape dimorphism? Just how much can natural selection and sexual selection be teased apart?

We are just beginning to test the questions about the role evolutionary history plays in patterns of sexual shape dimorphism. How do phylogenetic relationships affect sexual shape dimorphism? What role does sexual shape dimorphism play in microevolutionary patterns and what are the mechanisms underlying these patterns? What might result when these patterns are scaled from micro- to macroevolution? One way to address these questions is to take a sequential comparative approach: first examining patterns of dimorphism in two closely related species, then scaling up to

family, genera, and so forth. It is now also possible to ask if rates of evolution differ between species and if these rates differ more broadly between different sexually dimorphic traits. What effect do habitat and environmental gradients play in assessing rates and patterns of sexual shape dimorphism evolution? By examining the possible correlation between sexual shape dimorphism and habitat variables in a phylogenetic manner, it is possible to quantify hypotheses such as these. With the advent of new phylogenetic techniques, morphometric methods, and statistical testing, we can further examine the details of the evolution of sexual shape dimorphism.

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CHAPTER 3

BILL SHAPE AND SEXUAL SHAPE DIMORPHISM BETWEEN TWO SPECIES OF TEMPERATE HUMMINGBIRDS: BLACK-CHINNED HUMMINGBIRD (*ARCHILOCHUS ALEXANDRI*) AND RUBY-THROATED HUMMINGBIRD (*A. COLUBRIS*)

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Abstract

Sexual size dimorphism occurs throughout the animal kingdom, and its ecological and evolutionary causes and implications have been intensively studied. Sex-specific differences in bill curvature are known in several species of birds, including some tropical hummingbirds. Despite the importance of bill shape for foraging, comparative studies of sexual dimorphism of bill shape are few. We quantified bill shape in two temperate hummingbird species, Black-chinned Hummingbird (*Archilochus alexandri*) and Ruby-throated Hummingbird (*A. colubris*) and compared patterns of sexual shape dimorphism. Several commonly used bill-curvature indices yielded contrasting results; one found differences between species and sexes, a second identified no differences in curvature, and a circle-curvature approach revealed shape differences between species and between the sexes.

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By contrast, landmark-based geometric morphometric methods identified significant differences in sexual shape dimorphism and also revealed that Ruby-throated Hummingbirds exhibited significant sexual differences in shape, whereas Black-chinned Hummingbirds did not. Female Ruby-throated Hummingbirds exhibited relatively greater bill curvature than males, a pattern consistent with observations of some tropical hummingbirds. Although the causes of differences in bill-shape dimorphism between Black-chinned and Ruby-throated hummingbirds remain unclear, we hypothesize that it may be attributable to differences in the structure of the community in which each species breeds and the interplay between inter- and intraspecific competition for resources in these communities. Finally, we recommend that future studies of bill shape include geometric morphometric approaches because they are better suited than univariate approaches for identifying more complex shape differences within and among species.

Introduction

Sexual dimorphism is found throughout the animal kingdom, and its consequences for the ecology and evolution of organisms are often profound. One pattern frequently observed is sexual size dimorphism (SSD), and a number of mechanisms have been proposed to explain its variable distribution among taxa (Abouheif and Fairbairn 1997, Colwell 2000). Darwin (1871) proposed that sexual selection and selection for increased fecundity could be mechanisms that explain patterns of size dimorphism (see also Møller 1988, Andersson 1994). Other hypotheses suggest that sexual dimorphism evolves from competition over resources between the sexes (Selander 1972) or because

of sex-specific responses to environmental gradients (Hendry et al. 2006). In some species, sexual dimorphism may be driven by differences in the reproductive roles of males and females (Ralls 1976). When these processes occur in closely related species, distinct patterns of size dimorphism among species can result (Rensch 1960, Dale et al. 2007).

In addition to body size, males and females may also differ widely in shape (Hendry et al. 2006, Butler et al. 2007). Curiously, although shape contributes meaningfully to various ecological functions, such as feeding, mating, parental care, and other life- history characteristics, sexual shape dimorphism has received considerably less attention than sexual size differences (Lande and Arnold 1985, Hedrick and Temeles 1989, Gidaszewski et al. 2009). Sexual shape dimorphism has been identified in numerous taxa, including dipterans (Bonduriansky 2007), fish (Hendry et al. 2006), turtles (Valenzuela et al. 2004), and lizards (Butler et al. 2007), to name a few. In those cases that have been studied, both natural and sexual selection have contributed to the evolution of shape differences between the sexes (Hendry et al. 2006, Butler et al. 2007).

Hummingbirds are an ideal system for the study of sexual shape dimorphism. This diverse group of New World birds represents a monophyletic lineage (family Trochilidae) of 331 currently recognized species (McGuire et al. 2007). Both intraspecific and interspecific competition are prevalent in hummingbirds, both within and between the sexes (Kodric-Brown and Brown 1978). Females perform all parental care (Kaufman 1996), and migratory hummingbirds exhibit sexual differences in the pattern and timing of migration. Sexual dimorphism occurs in a number of

characteristics, such as wing morphology (Stiles et al. 2005) and, in some cases, bill morphology. Although many anatomical attributes contribute to successful foraging in hummingbirds, bill morphology is a critical trait that is likely under strong selection (Colwell 2000) and is tightly linked with feeding efficiency and foraging preferences in many species (Gould 1861, Darwin 1871). Bill morphology affects niche differentiation both among species and between the sexes (e.g., Feinsinger and Colwell 1978).

Considerable research has examined the functional relationship between hummingbird bill morphology and a species' food resource niche, particularly in tropical species (Snow and Snow 1972, Wolf et al. 1976, Feinsinger and Colwell 1978, Temeles and Roberts 1993, Altshuler and Clark 2003). Additionally, in some species, differences in bill size and bill curvature between the sexes are associated with differences in foraging (Paton and Collins 1989; Carpenter et al. 1991; Temeles et al. 2000, 2005, 2009). Hummingbirds also feed on arthropods, but foraging niches and bill morphology appear to be more related to nectivory (Stiles 1995). For example, the bills of female Purple-throated Caribs (*Eulampis jugularis*) are longer and more curved than the bills of males, and these bill differences correspond to morphological differences in the flowers (*Heliconia* spp.) that each sex primarily feeds on (Temeles et al. 2000, Temeles and Kress 2003).

Previous descriptions of hummingbird bill morphology have used univariate indices that were derived mainly from the ratios of linear measurements. One approach estimates bill curvature from measurements on the maxilla, whereas another estimates bill curvature from measurements of the mandible (e.g., Paton and Collins 1989, Stiles 1995). In addition, a recent approach used the mathematical definition of curvature to

quantify bill shape (Temeles et al. 2009). Although these methods provide simple and intuitive estimates of bill shape and curvature, several shortcomings may limit their utility for comparative studies across species. For instance, with the first two indices, it is possible that bills with different shapes can yield identical values, because the locations of the measurements on the bill are not recorded. For example, if maximum bill depth is nearer to the bill tip in one species and nearer to the base of the bill in another species, the ratio of length to depth could be the same for two species with different bill shapes (for a similar discussion, see Adams et al. 2004). Additionally, the third index assumes that bill curvature is constant across the length of the bill, because the approach is derived from the mathematical definition of curvature based on a circle. Although bill curvature may be relatively constant in some species (e.g., Purple-throated Carib), in others the curvature is greater either toward the tip (e.g., White-necked Jacobin [*Florisuga mellivora*]) or the base of the bill (e.g., Sword-billed Hummingbird [*Ensifera ensifera*]). In these cases, comparative studies among species may be compromised if univariate indices are used to quantify bill shape.

By contrast, landmark-based geometric morphometric methods (Rohlf and Marcus 1993, Adams et al. 2004) do not suffer from these shortcomings. These methods allow a rigorous quantification of shape from the locations of anatomical coordinates, after the effects of nonshape variation have been mathematically held constant.

Landmark-based morphometric methods are commonly used in evolutionary biology and anthropology to quantify shape differences in a wide variety of organisms (e.g., Adams and Rohlf 2000, Koecher et al. 2002, Kassam et al. 2003, Langerhans and DeWitt 2004,

Taylor et al. 2006, Butterworth et al. 2009), including birds (e.g., Foster et al. 2008, Kulemeyer et al. 2009, Navarro et al. 2009).

Two sister taxa of temperate-zone-breeding, migratory species, the Black-chinned Hummingbird (*Archilochus alexandri*) and the Ruby-throated Hummingbird (*A. colubris*), present an opportunity to compare bill shape across closely related taxa. The Black-chinned Hummingbird is a generalized and adaptable species (Baltosser and Russell 2000) whose breeding range overlaps that of several other species of hummingbirds in western North America (Ewald and Bransfield 1987). The Ruby-throated Hummingbird, on the other hand, is the only species of hummingbird that breeds in eastern North America (Robinson et al. 1996). Breeding territories of male and female Black-chinned Hummingbirds are found in different habitats (e.g., Stiles 1973, Baltosser 1989), whereas both sexes of the Ruby-throated Hummingbird share the same habitat. Finally, bill morphology in Ruby-throated Hummingbirds appears to be related to foraging and is correlated with flower morphology (Bertin 1982). Viewing these patterns in light of what is known of tropical hummingbirds suggests the hypothesis that bill morphology in these species may be shaped by foraging behavior and that morphological patterns of bill shape may differ between the sexes. To date, however, this hypothesis has not been formally tested.

We examined patterns of bill shape in Black-chinned and Ruby-throated hummingbirds to quantify interspecific differences in bill morphology as well as patterns of sexual shape dimorphism. From previous ecological work, we hypothesized that Ruby-throated Hummingbirds would exhibit greater sexual shape dimorphism than Black-

chinned Hummingbirds. We tested this hypothesis using bill shape quantified by the three curvature indices described above as well as by landmark-based geometric morphometric methods. In addition, we explicitly addressed the suggestion of Temeles and Kress (2003) that, although some hummingbirds have less extreme sexual size differences, they may still exhibit other sexual differences in bill morphology. If such patterns are identified, they suggest that foraging ecology plays a greater role in contributing to sexual differences across taxa than was previously appreciated.

Methods

Specimen information.—We obtained 286 adult Black-chinned Hummingbirds (160 females and 126 males) and 78 Ruby-throated Hummingbirds (35 females and 43 males) that were collected between 15 May and 15 August (see Appendix). The specimens were divided into geographic localities that corresponded to breeding populations. Our data thus consisted of Black-chinned Hummingbirds from two localities (Arizona and California) and Ruby-throated Hummingbirds from three localities (Michigan, New York, and Pennsylvania).

Morphometrics.—Bill shape of all specimens was measured using several approaches. First, we used three indices designed to capture overall bill curvature. One index estimates bill curvature by calculating the arc:chord ratio as the linear measurement of the exposed culmen (chord) to the curvilinear measurement (arc) taken across the maxilla (Stiles 1995; Fig. 1A). A second index measures curvature of the mandible as depth:length ratio from the linear distance of the mandible to gape (length) to the maximum distance between this measurement and the edge of the upper mandible

(height/gape, *sensu* Paton and Collins 1989, Collins 2008; Fig. 1B). The third index estimates bill curvature from the angle of declination of the bill in relation to the horizontal plane, using the mathematical definition of curvature as based on a circle (see Temeles et al. 2009; Fig. 1C). Finally, we used landmark- based geometric morphometric methods to quantify bill shape. First, digital images of the left-lateral side of the head and bill of each specimen were taken using a Nikon DXM-1200 digital camera mounted on a Nikon SMZ 1500 stereomicroscope. We then recorded the locations of 10 biologically homologous landmarks and 15 sliding semi-landmarks, which together were chosen to represent the shape and outline of the bill (Fig. 1D) using the program TPSDIG2 (Rohlf 2006). Nonshape variation was removed using a generalized Procrustes analysis that superimposed specimens onto a common coordinate system after accounting for differences in position, orientation, and scale (Rohlf and Slice 1990). During this procedure, semi-landmarks were permitted to slide along the outline of the bill to minimize shape differences (e.g., Bookstein et al. 1999). From the aligned specimens, shape variables were then generated using the thin-plate spline (Bookstein 1991) and standard uniform components (Rohlf and Bookstein 2003). Because the number of shape variables (46) was greater than the number of dimensions of actual shape information (due to the additional standardization of the semi-landmarks), we performed a principal component analysis (PCA) of shape and retained only the dimensions that contained variation. These 35 variables were then used in all subsequent analyses to represent bill shape. These procedures were performed in TPSRELW (Rohlf 2007).

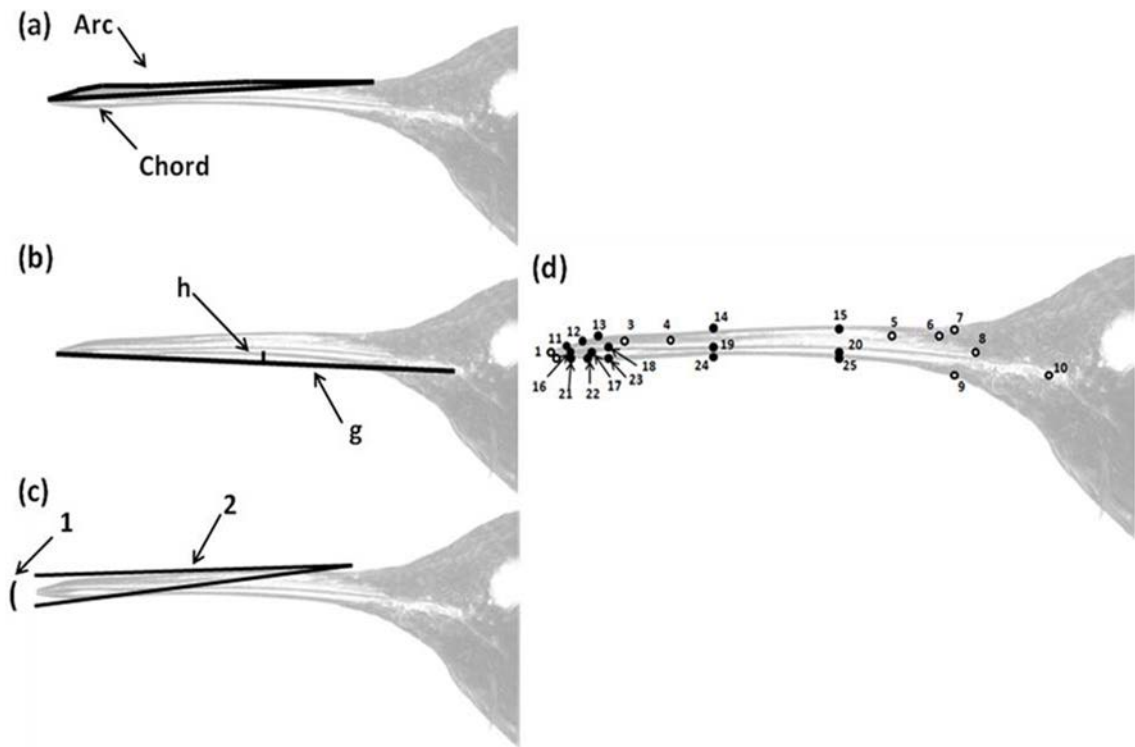


Figure 1. Curvature indices and landmark-based morphometrics, shown on a Black-chinned Hummingbird. (A) Measurements of the maxillary curvature index (arc:chord ratio). (B) Mandibular curvature index, where h is the maximum height of the edge of the upper mandible to g (bill tip to gape). (C) Circle-curvature method, where 1 is the angle and 2 is chord length. (D) Landmark-based geometric morphometrics. Open circles designate landmarks, and filled circles represent sliding semi-landmarks.

Nonshape variation was removed using a generalized Procrustes analysis that superimposed specimens onto a common coordinate system after accounting for differences in position, orientation, and scale (Rohlf and Slice 1990). During this procedure, semi-landmarks were permitted to slide along the outline of the bill to

minimize shape differences (e.g., Bookstein et al. 1999). From the aligned specimens, shape variables were then generated using the thin-plate spline (Bookstein 1991) and standard uniform components (Rohlf and Bookstein 2003). Because the number of shape variables (46) was greater than the number of dimensions of actual shape information (due to the additional standardization of the semi-landmarks), we performed a principal component analysis (PCA) of shape and retained only the dimensions that contained variation. These 35 variables were then used in all subsequent analyses to represent bill shape. These procedures were performed in TPSRELW (Rohlf 2007).

Statistical analyses.—We used analysis of variance (ANOVA) to examine variation in bill morphology. A factorial ANOVA was performed on each curvature index, with species and sex as main effects, and included a species*sex interaction term. For the landmark-based shape variables we used a factorial multivariate analysis of variance (MANOVA), with the same effects (species, sex, and species*sex interaction), to determine whether species exhibited differences in bill shape and whether sexual shape dimorphism existed. We then determined whether patterns of sexual shape dimorphism were concordant across species, by comparing the observed morphological differentiation between male and female Ruby-throated Hummingbirds with that exhibited in Black-chinned Hummingbirds.

For the landmark-based shape variables, the observed sexual shape dimorphism of each species was expressed as a multivariate vector that connected the phenotypic means of males and females (see Adams and Collyer 2009). Differences in the magnitude of these vectors were considered a measure of the difference in sexual shape dimorphism

between species. We calculated sexual shape dimorphism vectors for each species and performed a residual randomization to statistically evaluate difference in sexual shape dimorphism (see Hollander et al. 2006; Adams and Collyer 2007, 2009; Collyer and Adams 2007). We compared the observed difference in vector magnitudes with an empirically generated random distribution (from 9,999 iterations) obtained by permuting residuals from a reduced model that contained only main effects. For factorial designs, this procedure has superior statistical power to alternative randomization procedures (Anderson and ter Braak 2002).

To determine whether patterns of sexual shape dimorphism were consistent across localities within each species, we performed a MANOVA in which locality and sex were main effects, with a locality*sex interaction term. A separate MANOVA was performed on each species. Patterns of sexual shape dimorphism were then compared across localities, using the vector approach described above. Additionally, we performed a multivariate analysis of covariance with centroid size as a covariate and compared the fit of a model that incorporated size (species*sex*size) to a model without size (species*sex) (e.g., Gidaszewski et al. 2009) using Akaike's information criterion (AIC). All statistical analyses were performed in R (R Development Core Team 2008).

Finally, patterns of shape variation were graphically depicted in a principal component plot, with vectors of sexual shape dimorphism included. Thin-plate spline deformation grids were also generated for phenotypic means of males and females to facilitate biological interpretation of the observed shape differences within and between species.

Results

No differences in bill curvature were identified between species or the sexes when we used the maxillary curvature index (Table 1A). By contrast, significant differences between species and the sexes were found with the mandibular index (Table 1B) and the circle- curvature index (Table 1C).

Table 1. Statistical results from linear measurements of (A) the maxillary curvature index, (B) the mandibular index, and (C) the circle-curvature method. Significant effects are shown in bold.

Source	Mean squares	Approximate F	df	<i>p</i>
<i>(A) Maxillary curvature index: Arc:chord ratio</i>				
Species	4.0 E-7	1.222	1, 359	0.2902
Sex	2.3 E-5	0.02	1, 359	0.8877
Species*sex	2.5 E-5	1.221	1, 359	0.2698
<i>(B) Mandible curvature index: height/gape</i>				
Species	0.0033	33.6665	1, 359	<0.0001
Sex	0.0014	14.7949	1, 359	<0.0001
Species*sex	0.0001	0.0403	1, 359	0.8409
<i>(C) Circle-curvature: 1/radius</i>				
Species	1.2 E-5	17.3115	1, 359	<0.0001
Sex	3.0 E-5	6.6159	1, 359	<0.0001
Species*sex	4.1 E-5	23.9225	1, 359	<0.0001

With the latter approach, female Black- chinned Hummingbirds were predicted to have more curved bills than males ($K_{males} = 0.0043$, $K_{females} = 0.0053$), whereas this pattern was reversed in Ruby-throated Hummingbirds ($K_{males} = 0.0050$, $K_{females} = 0.0040$).

Using landmark-based morphometric methods, we identified bill-shape

differences between species, between the sexes, and in the interaction between species and sex (Table 2A). The degree of sexual shape dimorphism thus differed between the species (Table 2B, C). Separate within-species analyses confirmed this finding, identifying significant sexual shape dimorphism in Ruby-throated Hummingbirds but not in Black-chinned Hummingbirds (Table 2B, C).

Table 2. Statistical results from landmark-based geometric morphometrics quantifying (A) between-species morphological variation and (B, C) within-species morphological variation in Black-chinned Hummingbirds and Ruby-throated Hummingbirds. Significant effects are shown in bold.

Source	Pillai's trace	Approximate F	df	P
<i>(A) Between species</i>				
Species	0.6245	15.4193	35,325	<0.0001
Sex	0.1433	1.5576	35,325	0.0266
Species*sex	0.1491	1.6317	35,325	0.0161
<i>(B) Within Black-chinned Hummingbirds</i>				
Population	0.2018	1.7919	35,247	0.0059
Sex	0.1552	1.3019	35,247	0.1294
Population*sex	0.4425	0.0403	35,247	0.7083
<i>(C) Within Ruby-throated Hummingbirds</i>				
Population	1.3427	2.2764	35,38	<0.0001
Sex	0.6146	1.7313	70,78	0.0498
Population*sex	0.9122	0.9345	70,78	0.6123

Additionally, size did not explain this pattern, given that a model that included size as a covariate did not provide a better overall fit than the model that included species and sex alone ($AIC_{\text{species,sex}} = -138,574.4$ vs. $AIC_{\text{species,sex,size}} = -138,701.5$). Thus, allometric effects were relatively less influential on bill shape than the effects of species and sex.

Ruby-throated Hummingbirds exhibited a significant four- fold-greater degree of

sexual shape dimorphism than Black-chinned Hummingbirds ($D_{\text{Ruby-throated}} = 0.0227$, $D_{\text{Black-chinned}} = 0.00521$, $P = 0.004$), a difference that was evident when viewed using PCA (Fig. 2).

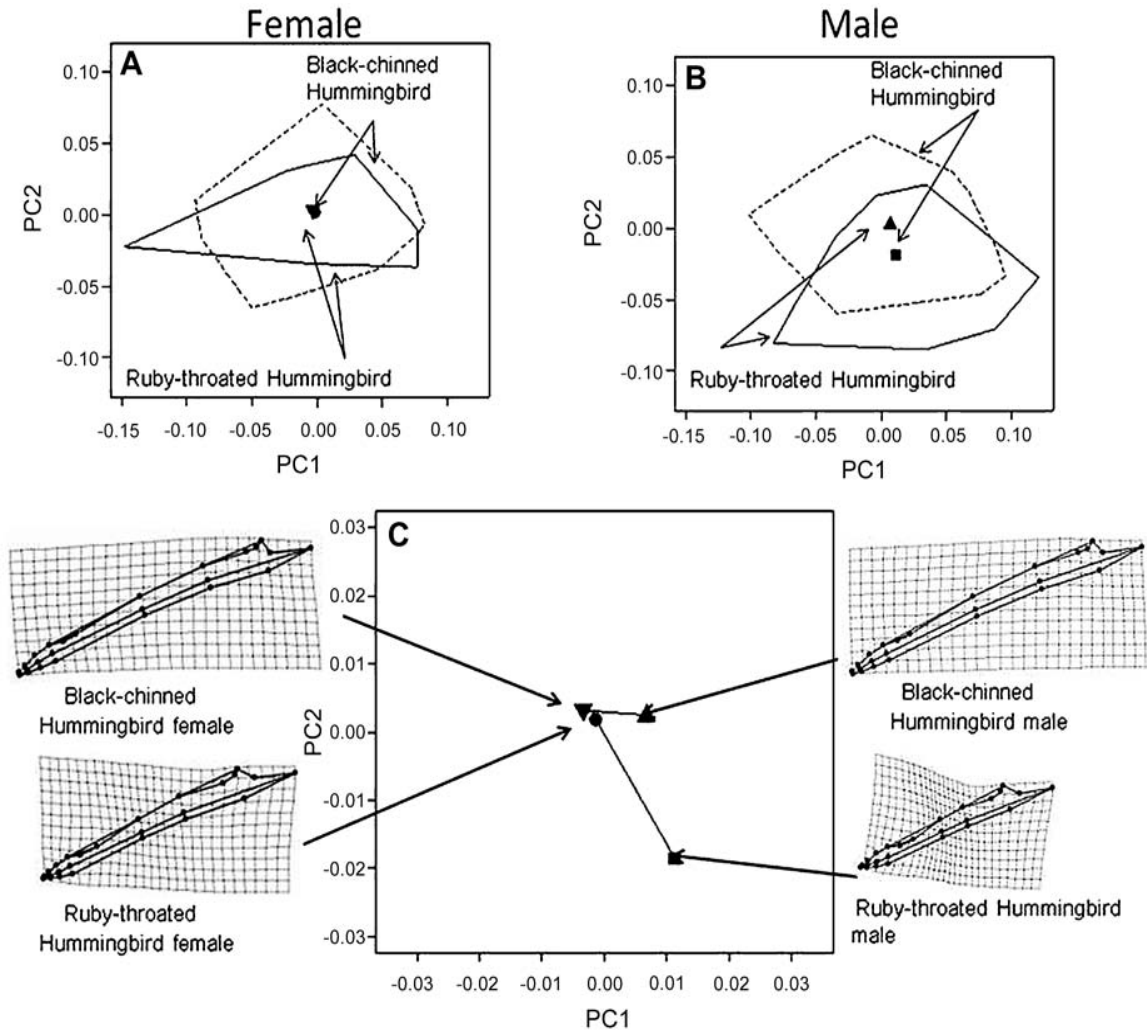


Figure 2. Results of principal component analysis of all specimens (relative warp scores). Group means are displayed in each plot for the first two principal components (PC1 = 36.9% and PC2 = 22.9%, accounting for 59.8% of the overall shape variation). Convex hulls in PCA plot (A and B) are displayed with dashed lines to represent Black-chinned Hummingbirds, and solid hulls represent Ruby-throated Hummingbirds. (C) Thin-plate spline deformation grids are shown to visualize patterns of shape variation and have been accentuated by a factor of 3 to enhance interpretation.

Further, visualization of bill shape with thin-plate spline deformation grids made these differences even more apparent (Fig. 2C). Specifically, the bills of Ruby-throated Hummingbirds were shorter, deeper, and stouter than the curved and elongated bills of Black-chinned Hummingbirds. And though the bills of Black-chinned Hummingbirds were relatively more curved than those of Ruby-throated Hummingbirds, both males and females in this species displayed similar bill shapes (Fig. 3A). By contrast, the significant sexual shape dimorphism in Ruby-throated Hummingbirds was such that bills of females were longer and more curved than the shorter and stouter bills of males (Fig. 3B).

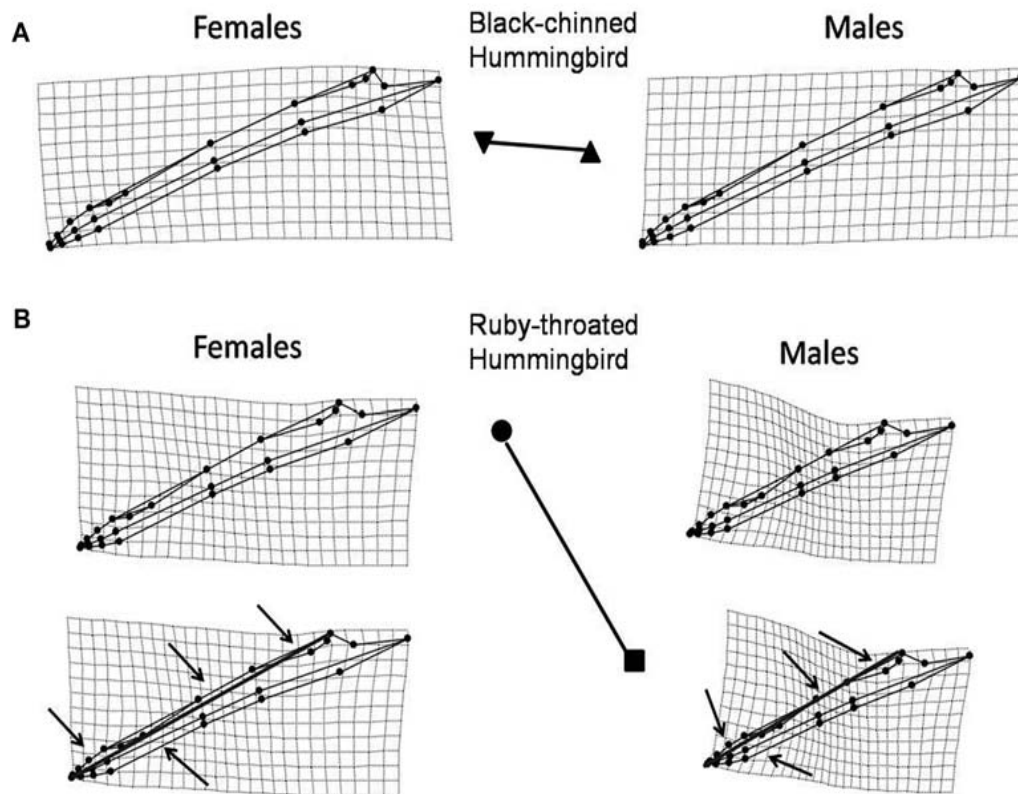


Figure 3. Thin-plate spline deformation grids and magnitude of sexual shape dimorphism in (A) Black-chinned Hummingbirds and (B) Ruby-throated Hummingbirds. Arrows accentuate the patterns of bill shape in relation to the straight line drawn from the tip to the exposed culmen to accentuate specific shape differences between the sexes. Deformation grids are scaled to a factor of 3 to enhance interpretation.

Interestingly, though the greater curvature in female Ruby-throated Hummingbirds is readily apparent in the graphic visualizations and is reflected in the landmark analyses, this pattern is not captured by the curvature index, which estimated females as having relatively less curvature in this species than males. We attribute this difference between patterns to the fact that bill curvature in this species is not constant across the length of the bill.

Discussion

For over a century, biologists have examined patterns of sexual dimorphism and the possible mechanisms responsible for its evolution. In hummingbirds, sexual dimorphism in plumage, physiology, behavior, and bill size have been documented, and though some studies have demonstrated the importance of differences in bill curvature between the sexes, fewer studies have focused on sexual shape dimorphism. Here, we examined bill-shape differences between the temperate sister taxa Black-chinned and Ruby-throated hummingbirds and tested the hypothesis that patterns of sexual dimorphism in bill shape differed between these species. Using a maxillary curvature index, we detected no differences in bill morphology between species or sexes. By contrast, both a mandibular curvature index and a circle-curvature approach revealed morphological differences between the two species and the sexes. When bill shape was quantified using landmark-based geometric morphometric methods, we found significant differences between species and identified significant differences in shape dimorphism between the sexes in Ruby-throated Hummingbirds but not in Black-chinned

Hummingbirds. This latter result revealed that sexual shape dimorphism was species-specific.

One interesting finding of our study is that different measures of bill curvature produced divergent results. This is important, because it may cause researchers who employ alternative approaches to draw different conclusions from the same data. Some of these differences may be explained by the fact that these methods quantify different anatomical regions: one quantifies maxillary curvature whereas another quantifies mandible curvature. A third method assumes constant bill curvature, which is clearly not the case in the species examined here (Fig. 3). Landmark-based geometric methods are less constrained than the other methods and have proved capable of identifying shape differences in this and other systems. The fact that we found differences attributable to methodology should draw attention to the fact that some aspects of bill shape are not captured by the univariate curvature indices. We therefore recommend that future studies quantify bill shape using landmark-based geometric morphometric methods.

Another finding of our study is that closely related taxa can display differing degrees of sexual shape dimorphism. Why would we expect differences in sexual shape dimorphism among related taxa? One possible explanation is that bill shape is influenced by sexual selection. The strength of sexual selection differs among species, and therefore the degree of dimorphism between the sexes might be expected to vary as well (Møller 1988, Andersson 1994, Owens and Hartley 1998). An alternative explanation is that sex-specific divergent selection occurs through differential responses to environmental gradients by each sex (Hendry et al. 2006). If such sex-specific responses differed among

species, this process would result in varying degrees of sexual shape dimorphism as well. Finally, sexual dimorphism may be accentuated in species-poor communities, where members of each sex may inhabit ecological niches occupied by interspecific competitors in species-rich communities (Butler et al. 2007). Patterns consistent with this hypothesis have been identified in a number of vertebrate taxa (Schoener 1967, Dayan and Simberloff 1994) and may enhance the process of adaptive radiation in some circumstances, as sexes evolve different adaptations to resources and occupy different regions of niche space and morphospace (Butler et al. 2007).

Patterns of sexual shape dimorphism in bill shape are not unusual. In birds, it is relatively common for males and females of the same species to specialize on different trophic resources (Selander 1966), and in some species, sex-specific trophic differences are related to differences in bill morphology. For example, the tropical Purple-throated Carib exhibits bill-shape differences between the sexes that correspond to morphological differences in the *Heliconia* flowers that each sex primarily feeds on (Temeles et al. 2000, 2009; Temeles and Kress 2003). Sexual dimorphism in bill shape and resource use are also found in other avian species, such as the extinct Huia (*Heteralocha acutirostris*) of New Zealand (Burton 1974) and the African Green Woodhoopoe (*Phoeniculus purpureus*; Jamieson and Spencer 1996, Radford and du Plessis 2003), among others.

Although the reason(s) for the difference in sexual shape dimorphism in the two species that we studied is unclear, we hypothesize that differences in the breeding behavior of the two hummingbird species may be a contributing factor. Male and female Black-chinned Hummingbirds hold territories in different habitats, whereas male and

female Ruby-throated Hummingbirds hold territories in the same habitat during the breeding season. It is likely that selection is strongest during the breeding period because it is the most energetically expensive time for hummingbirds: males exhibit costly courtship displays and territory defense (Stiles 1971, Armstrong 1987) and females must perform all parental care. We further hypothesize that the pattern of sexual shape dimorphism may also be attributable to “ecological release” (e.g., Selander 1966). Under this scenario, the lack of potential competitors in the breeding range of Ruby-throated Hummingbirds may allow expansion of its ecological niche that results in morphological differences between the sexes. In the Copper-rumped Hummingbird (*Amazilia tobaci*), for example, a broader feeding niche and greater morphological variation are observed in populations that co-occur with few other hummingbird species, whereas both ecological and morphological variation are reduced in populations that are sympatric with many other competing species (Feinsinger and Swarm 1982). Our study reveals a similar pattern. The geographically isolated populations of Ruby-throated Hummingbirds (Robinson et al. 1996) display greater morphological differences between the sexes, whereas Black-chinned Hummingbirds display less morphological differentiation between the sexes and are found in communities that are sympatric with multiple hummingbird species (Ewald and Bransfield 1987). Thus, differences in sexual shape dimorphism may be attributable to the interaction between intra- and interspecific competition in different communities (*sensu* Dayan and Simberloff 1994; see Butler et al. 2007). If this hypothesis is correct, we predict that other hummingbird species that are sympatric with Black-chinned Hummingbirds would also have reduced levels of sexual

shape dimorphism compared with Ruby-throated Hummingbirds. We emphasize, however, that for these species, the biological causes of differential sexual shape dimorphism, such as variable community structure (Butler et al. 2007), sexual selection (Møller 1988, Andersson 1994), and differential responses to environmental gradients (Hendry et al. 2006), have yet to be fully explored.

We found that female Ruby-throated Hummingbirds have longer and more curved bills than males, although this is less dramatic than in Purple-throated Caribs (Temeles et al. 2000, 2005, 2009; Temeles and Kress 2003). Studies of the two species thus support Brown and Kodric-Brown's (1979) prediction that sexual differences in bill morphology should be greater in tropical than in temperate hummingbird species. Greater difference in the magnitude of sexual shape dimorphism in tropical than in temperate hummingbird species may be attributable to the widely distributed and morphologically similar flower resources that are available to temperate hummingbirds. The much greater diversity of floral species and floral forms in the tropics (Grant and Grant 1968) must surely contribute to greater shape variation at low latitudes. Examination of the difference in the magnitude of sexual shape dimorphism in a wider array of taxa will make it possible to address how patterns of sexual shape dimorphism evolve across the landscape and to evaluate the extent to which sexual shape dimorphism is associated with the environmental niche of each species. When viewed in a broader context, ecological examinations of sexual shape dimorphism must also be viewed in light of phylogenetic history to fully understand the interplay between bill-shape sexual dimorphism and resource use and the coevolution of these two suites of traits.

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Appendix

Specimens examined to quantify morphological variation:

The left lateral side of hummingbird bills from collections at Carnegie Museum of Natural History (CMNH), Cornell University Museum of Vertebrates (CUMV), Delaware Museum of Natural History (DMNH), Field Museum of Natural History (FMNH), Los Angeles County Museum (LACM), Museum of Comparative Zoology (MCZ), Museum of Southwestern Biology (MSB), Museum of Vertebrate Zoology (MVZ), San Diego Natural History Museum (SDNHM), University of Michigan Museum of Zoology (UMMZ), National Museum of Natural History (NMNH), Western Foundation of Vertebrate Zoology (WFVZ), and Yale Peabody Museum (YPM).

Specific specimens, listed by institution, include:

CMNH 125705, 125706, 125710, 141875, 142477, 151407, 152912, 153151, 153802, 154036, 154146, 154621, 158828, 1588289, 158830, 158831, 158832, 158833, 158834, 158836, 158837, 159323, 159324, 159325, 159327, 159328, 159329, 159330, 159331, 159332, 159333, 159334, 159335, 159336, 159337, 159338, 159339, 159340, 159342, 159343, 159345, 159346, 159347, 159348, 159349, 159350, 166922, 167457, 168761, 169649, 170846, 170867, 95217

CUMV 14986, 21604, 23717, 24397, 37009, 44079, 44128, 44129, 44130, 44131, 44171, 49013, 50001, 50780, 51239, 5352, 5355, 5357, 5358, 5361, 5362, 5363, 5364, 5365, 5367, 5382

DMNH 1400, 1426, 1436, 19019, 19025, 59806

FMNH 138705, 138720, 138723, 138725, 14653, 14654, 14655, 14656, 14657, 14658, 14659, 14661, 14662, 14663, 14666, 14667, 14668, 14670, 14672, 14673, 14674, 14675, 14676, 14678, 159838, 159839, 161019, 16575, 16576, 93036, 93037, 93038, 93039, 93040

LACM 14127, 14138, 14139, 1927, 3381, 78618, 78619, 78621, 78623, 78624, 78625, 78626, 78627, 78632, 78643, 78646, 78652, 78653, 78654, 78656, 78659, 78661, 78662, 78663, 78664, 78668, 78669, 78671, 78677, 78678, 78683, 78684, 78688, 78691, 78694

MCZ 100182, 103286

MSB 14801, 22663, 22665, 22668, 22670, 22671, 22677

MVZ 107024, 116741, 121811, 19920, 19921, 19922, 19923, 19925, 22484, 22704, 26729, 26730, 27927, 27928, 27929, 27930, 27931, 3192, 3194, 3197, 32864, 32870, 32875, 32878, 32880, 32881, 32882, 32883, 32884, 32887, 32888, 32889, 32900, 32903, 3655, 3698, 3700, 3785, 40727, 40728, 40731, 41534, 41927, 4194, 4195, 43242, 45343, 5249, 77338, 80946

NMNH 108369, 117258, 117269, 129247, 129249, 129250, 129252, 129257, 134343, 140254, 140255, 140256, 140257, 140258, 140259, 140266, 168422, 203268, 234334, 235077, 235074, 258387, 467031, 589963, 91738, 91740, 94957, 91959

SDNHM 17251, 22386, 22387, 22389, 22463, 30108, 30417, 449, 450

UMMZ 111820, 113646, 113649, 126291, 126292, 126294, 126295, 126296, 126297, 126299, 126300, 126301, 126301, 126302, 126303, 126304, 126306, 126307, 126310, 126314, 134697, 136434, 156250, 164455, 164461, 164463, 164468, 164470, 164471, 164472, 164473, 164474, 164490, 164491, 164492, 164492, 164496, 164498, 164500,

164501, 164502, 164504, 164505, 164506, 164507, 164508, 164509, 164510, 164514,
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WFVZ 10119, 1515, 1516, 1517, 21798, 21803, 21804, 21806, 21809, 2714, 32155,
32156, 49304, 49308

YPM 6442, 99650

CHAPTER 4**BECOMING DIFFERENT BUT STAYING ALIKE: PATTERNS OF SEXUAL SIZE AND SHAPE DIMORPHISM IN BILLS OF HUMMINGBIRDS**

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Abstract

Hummingbirds are known for their distinctive patterns of sexual dimorphism, with many species exhibiting sex-related differences in various ecologically-relevant traits, including sex-specific differences in bill shape. It is generally assumed that such patterns are consistent across all hummingbird lineages, yet many taxa remain understudied. In this study we examined patterns of sexual size and sexual shape dimorphism in bills of 32 of 35 species of the monophyletic *Mellisugini* lineage. We also compared patterns of bill size dimorphism in this group to other hummingbird lineages, using data from 219 hummingbird species. Overall, the presence and degree of sexual size dimorphism was similar across all hummingbird lineages, with the majority of *Mellisugini* species displaying female-biased sexual size dimorphism, patterns that remain unchanged when analyzed in a phylogenetic context. Surprisingly however, we found that sexual dimorphism in bill shape was nearly absent in the *Mellisugini* clade, with only 3 of the 32 species examined displaying bill shape dimorphism.

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Based on observations in other hummingbird lineages, the lack of sexual shape dimorphism in *Mellisugini* is particularly unusual. We hypothesize that the patterns of sexual size dimorphism observed here may be the consequence of differential selective forces that result from competition for ecological resources. We further propose that an influential mechanism underlying shape dimorphism is competition and niche segregation. Taken together, the evolutionary changes in patterns of sexual shape dimorphism observed in *Mellisugini* suggest that the evolutionary trends of sexual dimorphism in the *Trochilidae* are far more dynamic than was previously believed.

Introduction

Understanding the origin of phenotypic diversity is a major focus of evolutionary research, and patterns of sexual dimorphism represent a particularly intriguing component of this diversity. Sexual dimorphism is exhibited throughout the animal kingdom (see e.g., Butler et al. 2000) and is displayed in a myriad of ways, including sex-specific behavior and vocalizations (Väisänen et al. 2005), sexual differences in body size (Fairbairn 1997), coloration (Dryden and Mardia 1993), and other morphological traits (Berns and Adams 2010a). Darwin (1871) drew attention to these patterns and suggested that morphology can vary between the sexes due to the action of sexual selection operating in one or more ways, which subsequently enhances sex-specific fitness in relation to reproduction (Darwin 1871, Jones and Ratterman 2009b).

A number of mechanisms have been proposed to explain the evolution of sexual dimorphism. For example, sexual selection can generate sex-specific differences as the sexes evolve in distinct directions that maximize their own reproductive success (Darwin 1871, Andersson 1994). Alternatively, ecological mechanisms such as competition for resources, may exert distinct selective forces on the sexes, resulting in the evolution of sexual dimorphism (Selander 1972, Hedrick and Temeles 1989, Rohlf 1999). Here, intraspecific competition in species-poor communities may allow divergent selection between the sexes (rather than between species) to result in niche segregation between males and females (i.e., intersexual niche packing: *sensu* Butler et al. 2000). A third possible mechanism that may enhance sexual dimorphism in some species is the influence of sex-specific divergence in response to environmental gradients, where males and females exhibit differential responses to the same environmental selective pressures (Hendry et al. 2006b). For instance, in guppies (*Poecilia reticulata*), open canopy sites resulted in selection for smaller heads and distended abdomens in females but not in males, whereas both sexes in high flow sites had small heads and deeper caudal peduncles (Hendry et al. 2006). Likewise, weaker latitudinal clines in male houseflies (*Musca domestica*) but not in females suggest sex-specific responses to food resource abundance which may vary clinally (Stephens and Wiens 2009).

Hummingbirds (family *Trochilidae*) offer a unique opportunity to study patterns of sexual dimorphism and elucidate the underlying mechanisms responsible for these patterns. This monophyletic lineage of 338 currently recognized species (McGuire et al. 2007, McGuire et al. 2009) has been a model taxon for the study of sexual dimorphism,

as these species exhibit patterns such as dimorphic plumage (Darwin 1871, Bleiweiss 1992, 1997) sex-specific behavior (Feinsinger and Colwell 1978, Kodric-Brown and Brown 1978)), and ornamentation (Evans et al. 1995, Zusi and Gill 2009), among others. Several hypotheses have been proposed to explain the evolution of this sexual dimorphism in this group. For example, the remarkable ornamentation that males sometimes exhibit, such as the tail ‘boots’ of the hummingbird *Ocreatus underwoodii* (Booted Racket-tail) or tail length in *Phaethornis superciliosus* (Long-tailed Hermit) may be due to ‘Fisherian runaway selection’, where females prefer males that exhibit more extreme phenotypes within a population, which over evolutionary time become increasingly exaggerated despite the potential fitness costs to the males themselves (Andersson 1994). Further, differential plumage coloration between the sexes in some species is thought to be the result of females selecting males that exhibit superior characteristics (i.e., the ‘good genes’ hypothesis: *sensu* Darwin 1871, Bleiweiss 1992, 1997).

Hummingbirds also exhibit sexual dimorphism in both body size and bill morphology. For example, in hummingbirds bill morphology is under strong selection pressures due to its role in foraging, and differential foraging among species is thought to be a major cause of diversification in trophic structures among species (Feinsinger 1978, Brown and Bowers 1985, Collins and Paton 1989, Bleiweiss 1998). Additionally, males and females of some species forage differently (Carpenter et al. 1991, Temeles and Kress 2003) and many of these species also display sexual dimorphism in bill size and bill curvature (Temeles et al. 2010). Recently, quantitative support for Darwin’s hypothesis

is found in some species where there is a direct link between sexual differences in bill morphology in *Eulampis jugularis* (Purple-throated Carib) and patterns of sex-specific foraging. Here, males and females of this species forage on distinct resources, and the bill morphology of each sex is correlated with the morphology of the flower that each feeds from (the ‘ecological causation hypothesis’ *sensu* Temeles et al. 2010). Similar patterns have been observed in other clades of hummingbirds, where differences in bill sexual size and shape dimorphism have a direct link to feeding ecology (Temeles et al. 2010). Together, these findings suggest that, not only is sexual dimorphism in bill morphology the evolutionary result of various ecological processes and sex-specific adaptations to foraging (Temeles et al. 2010), but also that bill size and shape dimorphism is common throughout hummingbirds.

Much of the prior work on sexual dimorphism in *Trochilidae* has focused on species in tropical lineages known to display sexual dimorphism, and has focused largely on dimorphism in bill size and bill shape (Rodríguez-Flores and Stiles 2005, Temeles et al. 2010). Based on these studies, it has generally been assumed that sexually-dimorphic patterns in bill shape are consistent across *Trochilidae* due to the tight coevolutionary link between bill morphology and flower morphology in this group, yet little work on sexual dimorphism has been performed in taxa outside of a few focal lineages (e.g., Bleiweiss 1999; Temeles et al. 2005). For example, a few studies have examined sexual dimorphism in bill morphology in select species in the *Mellisugini* clade, finding sexual size dimorphism of the bill in *Selasphorus rufus* (Temeles and Roberts 1993), *S. scintilla* and *S. flammula* (Stiles 1983). However the prevalence of such patterns in bill

morphology remains largely unknown, as most of the 35 species in the *Mellisugini* clade have yet to be examined. Recently, it was observed that two sister species within the *Mellisugini* clade, *Archilochus alexandri* and *A. colubris*, differ in their patterns of dimorphism in bill morphology (Berns and Adams 2010a), where both species display significant bill size dimorphism, but only *A. colubris* exhibits bill sexual shape dimorphism. These results were surprising, as prior work on *Trochilidae* in other clades suggested that shape dimorphism in bill curvature is common (bill curvature is one aspect of bill shape: Temeles et al. 2010).

Given these findings, we conducted a broader study of species in the *Mellisugini* clade with the purpose of addressing the following questions. First, is sexual dimorphism in bill size common across species in the *Mellisugini* clade? Based on previous studies, we predicted that the majority of species in the *Mellisugini* clade would exhibit primarily female-biased (that is, the bill of females is larger than males) bill size dimorphism. Second, is the presence and pattern of sexual *shape* dimorphism in the *Mellisugini* clade concordant with those found across all *Trochilidae*? Studies of other clades have found sexual curvature dimorphism is common, therefore we predicted the same pattern would be present in the *Mellisugini* clade. We addressed these questions in a phylogenetic context using both linear measurements and landmark-based geometric morphometric techniques to quantify sexual size and shape dimorphism in the bill morphology in 32 of the 35 *Mellisugini* species (McGuire et al. 2009). We then compared patterns of bill size dimorphism in the *Mellisugini* lineage to those found in other *Trochilidae* lineages, using a dataset collected from prior studies published in the literature (219 species).

Methods

Specimen information.- We measured 1,347 hummingbirds representing 32 of the 35 species in the *Mellisugini* lineage, obtained from 14 museum collections (see Appendix). All specimens included in this study were adults of known sexes, and a roughly equal proportion of males and females of each species were examined (722 males, 625 females; see Appendix).

Morphometrics.-We obtained digital images of the left-lateral side of the head and bill of each specimen using a Nikon DXM-1200 digital camera mounted on a Nikon SMZ 1500 stereomicroscope (a Nikon D-90 was used to collect images obtained at museums, as the stereomicroscope system was not easily transportable). Each photograph included a ruler in order to account for size in the analyses, and the birds were placed in the same position on a modeled clay surface.

From these images, two sets of data were obtained. First, the exposed culmen was measured on each specimen (culmen length: CL: Fig. 1A; e.g., Bleiweiss 1999; Colwell 2000) and was treated as a measure of bill size for each individual. Culmen length and bill centroid size were highly correlated ($r = 0.97$), so only CL is used here as a measure of size. To place our findings in a broader phylogenetic context, we combined these linear measurements with additional bill length data that we obtained from the literature, yielding a total dataset of CL from 219 hummingbird species (see Appendix) across the family *Trochilidae*.

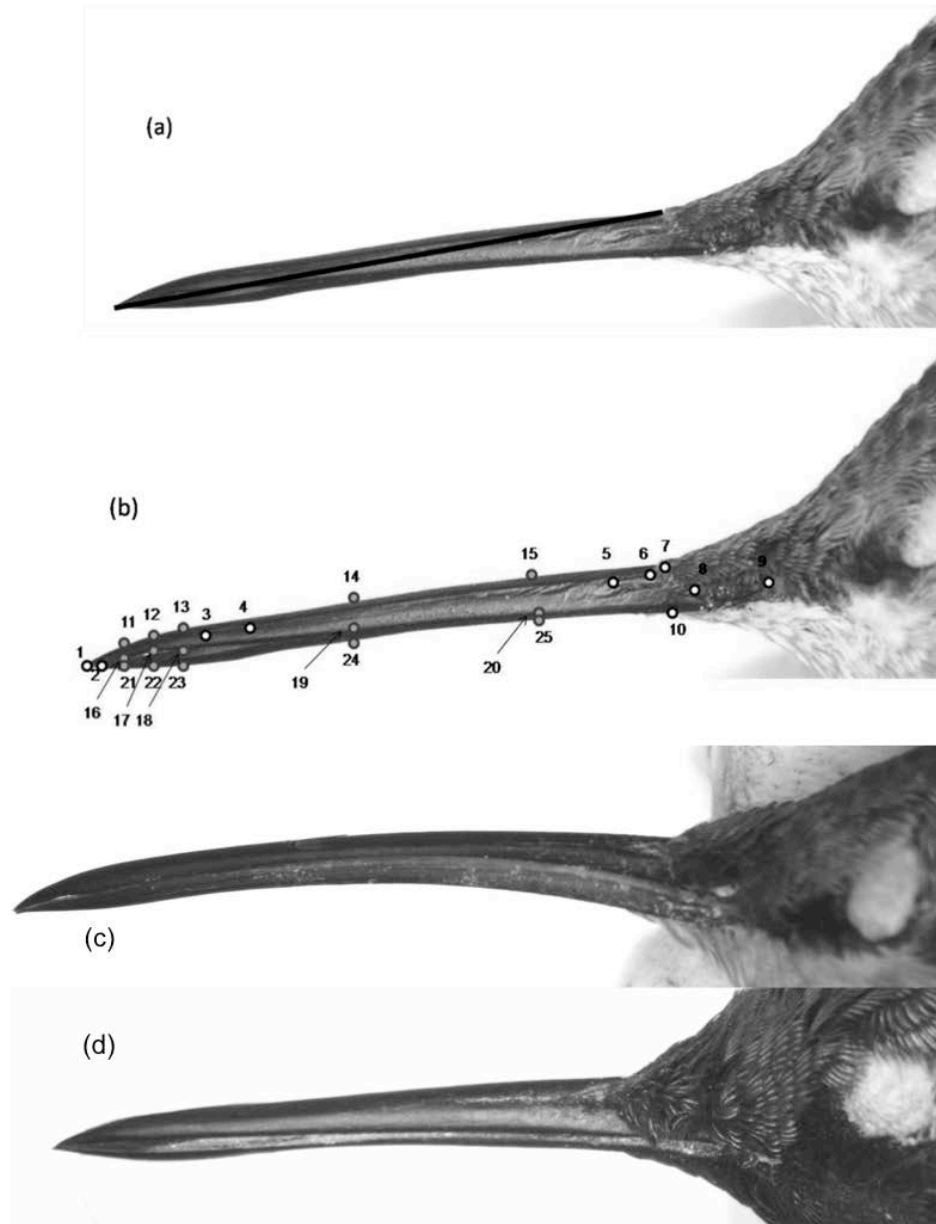


Figure 1. a) Exposed culmen representing bill length. b) Landmark-based geometric morphometrics. Open circles designate biologically homologous landmarks and filled circles represent sliding semilandmarks. c) Representative individual from the species with extreme bill curvature (*Calothorax lucifer*), and d) from the species with the straight bills (*Archilochus colubris*).

Second, bill shape was obtained from each image using landmark-based geometric morphometric methods (Rohlf and Marcus 1993, Adams et al. 2004). These methods allow for quantification of shape after the effects of non-shape variation (position, orientation, and scale) have been mathematically held constant. For this approach we first digitized the locations of 10 biologically homologous landmarks from the images of each specimen. Additionally, we included 15 sliding semilandmarks along the boundary curve of each bill to represent its shape and curvature (see Berns and Adams 2010a) using TPSDig 2 (Rohlf 2010).

Together, the 25 landmarks and semilandmarks were subjected to a Generalized Procrustes Analysis (GPA: Rohlf and Slice 1990). This least-squares procedure translates all specimens to the origin, scales them to unit centroid size, and optimally rotates them to minimize the total sums-of-squares deviations of the landmark coordinates from all specimens to the average configuration. During this procedure, semilandmarks were allowed to slide along their tangent directions (Bookstein et al. 1999, Gunz et al. 2005) so as to minimize Procrustes distance between specimens (see e.g., Serb et al. 2011). After superimposition, the aligned Procrustes shape coordinates describe the location of each specimen in a curved space related to Kendall's shape space (Rohlf 1999; Slice 2001). These were then projected orthogonally into a linear tangent space yielding Kendall's tangent space coordinates (Dryden and Mardia 1993; Dryden and Mardia 1998; Rohlf 1999), which were treated as a set of shape variables for further analyses of shape variation and covariation (e.g., Adams et al. 2007, Adams 2010a, Adams and Nistri 2010a).

Patterns of Sexual Size Dimorphism.- We performed two sets of analyses to assess patterns of sexual dimorphism of bill size. First, to determine whether bill size and size dimorphism differed among species in the *Mellisugini* clade, we used our culmen length data and a two-factor ANOVA; where variation in bill size (culmen length) was explained by species, sex, and their interaction. A significant species \times sex term would imply that there was a significant difference in the degree of sexual size dimorphism among species. Due to the large differences in variance within the species \times sex groups, we also ran ANOVAs for each species separately to determine whether sexual dimorphism was present in each species.

Second, we performed a family-wide analysis of bill size dimorphism, using the bill size measurements of the 32 species in the *Mellisugini* clade as above, as well measurements of an additional 187 species obtained from literature (Bleiweiss 1999, Colwell 2000, Temeles et al. 2010). In total, this analysis included 219 of 338 hummingbird species (Birdlife.org), or 65% of the current diversity of the group. From these measurements, the average male and female bill sizes (\overline{CL}_M , \overline{CL}_F), were obtained. Next, measures of sexual size dimorphism were estimated as the Lovich-Gibbons ratio (Lovich and Gibbons 1992), which is found as: $\left(\overline{CL}_F/\overline{CL}_M - 1\right) * 1$ for species where the female is the larger sex, and $\left(\overline{CL}_M/\overline{CL}_F - 1\right) * (-1)$ when the male is the larger sex (see Stephens and Wiens 2009; Temeles et al. 2010). Using these size-dimorphism ratios, we performed an ANOVA to determine whether the seven major hummingbird clades for which we had information (*sensu* McGuire et al. 2009) differed in their patterns of sexual size dimorphism. In addition, we performed a phylogenetic ANOVA (Garland et

al. 1993) on the same ratio data to account for non-independence due to shared evolutionary history. For this approach, the evolutionary relationships among species were based on the current molecular phylogeny for hummingbirds (McGuire et al. 2007). Finally, these analyses were repeated on the simple ratio of male to female bill size ($\overline{CL_M} / \overline{CL_F}$), from which statistically similar results were obtained (results not shown).

Patterns of Sexual Shape Dimorphism.— To determine whether bill shape and shape dimorphism differed among species in the *Mellisugini* clade we performed a two factor MANOVA, where variation in bill shape was explained by species, sex, and species \times sex interaction. A significant species \times sex term would imply that there were differences in the degree of sexual shape dimorphism among species. As with the ANOVA, we ran a separate permutational-MANOVA with 9,999 iterations (Anderson 2001) for each species to examine the degree of sexual shape dimorphism within each species. We then calculated vectors of sexual shape dimorphism for each species as the difference between male and female means (see Berns and Adams 2010), and used a permutation procedure with 9,999 iterations to determine whether the degree of sexual shape dimorphism differed between species (for details see Adams and Collyer 2007, 2009; Collyer and Adams 2007). Here, the observed sexual shape dimorphism for each species was quantified as the multivariate vector connecting male and female means for each species. The length of this vector (i.e., Euclidean distance) corresponds to the magnitude of sexual shape dimorphism exhibited by each species, which were compared

using the permutation procedure above to determine the extent to which the degree of sexual shape dimorphism differed among species (see Berns and Adams 2010a).

Visualization- Finally, to visualize patterns of bill shape variation, we performed a principal components analysis using the full set of Kendall's tangent space coordinates, and plotted the first two PCs, which described the largest amount of shape variation. In this principal components plot we also included vectors connecting male and female means for species that displayed significant sexual shape dimorphism (see Results below). We then generated thin-plate spline deformation grids (Bookstein 1991) for phenotypic means of males and females to graphically depict differences in bill shapes for these species, and to facilitate biological interpretation of the observed shape differences within and between them. All analyses were conducted in R version 2.11.1 (R Development Core Team, 2010), TpsSpline (Rohlf 2005) and TPSRelW (Rohlf 2004).

Results

Patterns of Sexual Size Dimorphism.—Using a two-factor ANOVA on the *Mellisugini* data, we found significant differences in bill size among species, between the sexes, as well as a significant species \times sex interaction term (Table 1A). The latter term revealed that the degree of sexual size dimorphism differed significantly among species. When sexual size dimorphism was examined for each species separately, we found that the majority of species (26) displayed significant sexual size dimorphism, while several other species did not (Table 1B).

Table 1. A) Statistical results from ANOVA on culmen length for 32 species of hummingbird in the *Mellisugini* lineage. B) ANOVA comparing male versus female culmen lengths for each of 32 species of *Mellisugini* hummingbirds. Size dimorphism ratios for each species (expressed as Lovich-Gibbons ratios) are shown. Significant effects are shown in bold.

A: Effects	Mean squares	Approx. F	df	P
Species	493.06	393.3193	31	<0.001
Sex	240.74	192.0392	1	<0.001
Species*Sex	2.66	2.1192	31	<0.001
Residuals	1.25		1283	

B: Species	Mean Sq	Approx. F	df	P	Ratio
<i>Archilochus alexandri</i>	33.165	19.070	1	<0.001	0.038
<i>Archilochus colubris</i>	62.972	125.479	1	<0.001	0.119
<i>Atthis Heliosa</i>	7.102	20.873	1	<0.001	0.086
<i>Calliphlox amethystina</i>	1.144	1.283	1	0.269	0.031
<i>Calliphlox bryantae</i>	3.936	6.626	1	0.017	0.053
<i>Calliphlox evelynae</i>	1.616	1.012	1	0.324	-0.008
<i>Calliphlox mitchelli</i>	6.236	9.152	1	0.005	0.069
<i>Calothorax lucifer</i>	2.958	2.585	1	0.119	0.032
<i>Calothorax pulcher</i>	25.477	10.599	1	0.003	0.123
<i>Calypte anna</i>	7.319	5.563	1	0.025	0.086
<i>Calypte costae</i>	4.664	5.128	1	0.032	0.051

Table 1B (<i>continued</i>): Species	Mean Sq	Approx. F	df	P	Ratio
<i>Chaetocercus astreans</i>	7.459	19.981	1	<0.001	0.082
<i>Chaetocercus bombus</i>	2.580	6.552	1	0.023	0.064
<i>Chaetocercus heliodor</i>	8.158	7.389	1	0.011	0.074
<i>Chaetocercus jourdanii</i>	5.939	6.592	1	0.016	0.067
<i>Chaetocercus mulsanti</i>	15.844	13.059	1	0.001	0.083
<i>Doricha eliza</i>	2.581	5.664	1	0.033	0.041
<i>Doricha enicura</i>	9.781	2.660	1	0.118	0.084
<i>Mellisuga helenae</i>	7.454	11.726	1	0.002	0.093
<i>Mellisuga minima</i>	5.451	14.684	1	0.001	0.091
<i>Microstilbon burmeisteri</i>	9.615	24.023	1	<0.001	0.047
<i>Myrmia micrura</i>	7.351	7.384	1	0.014	0.088
<i>Myrtis fanny</i>	0.293	0.197	1	0.660	-0.013
<i>Rhodopis vesper</i>	34.673	3.506	1	0.071	0.080
<i>Selasphorus flammula</i>	4.499	7.610	1	0.009	0.062
<i>Selasphorus platycercus</i>	11.400	8.092	1	0.009	0.074
<i>Selasphorus rufus</i>	14.766	36.033	1	<0.001	0.190
<i>Selasphorus sasin</i>	0.065	0.054	1	0.817	0.005
<i>Stellula calliope</i>	2.443	6.788	1	0.016	0.059
<i>Selasphorus scintilla</i>	4.896	5.838	1	0.021	0.056
<i>Thaumastura cora</i>	7.753	17.005	1	<0.001	0.060

Table 1B (<i>continued</i>): Species	Mean Sq	Approx. F	df	P	Ratio
<i>Tilmatura dupontii</i>	3.503	8.499	1	0.007	0.057

Interestingly, we identified considerable variation in the degree of size dimorphism exhibited among species in this group, with a 27-fold difference in the degree of sexual size dimorphism between the species with the least size dimorphism (*Selasphorus sasin*: sexual size dimorphism = 0.0045), and the most dimorphic species (*Calothorax pulcher*: sexual size dimorphism = 0.12257).

When magnitudes of sexual size dimorphism were examined across the entire hummingbird family, we found no difference in the degree of size dimorphism exhibited among clades within *Trochilidae* ($F_{7,211} = 0.632$; $P = 0.7292$), suggesting that all groups displayed a similar degree of size dimorphism. Similarly, no differences among clades were identified when the phylogenetic relationships among species were taken into consideration ($F_{7,114} = 0.2143$; $P_{\text{rand}} = 0.9714$). While all species displayed similar magnitudes of size dimorphism in their bills, overall the majority of species (156 of 219) were female-biased (Fig. 2). Consistent with prior results, some lineages (e.g., *Phaethornithinae*) displayed male-biased size dimorphism for the majority of their species (e.g., Temeles et al. 2010: Fig. 2). However, this was not the case in the *Mellisugini* clade, where 92% of the species examined exhibited female-biased size dimorphism (Fig. 2).

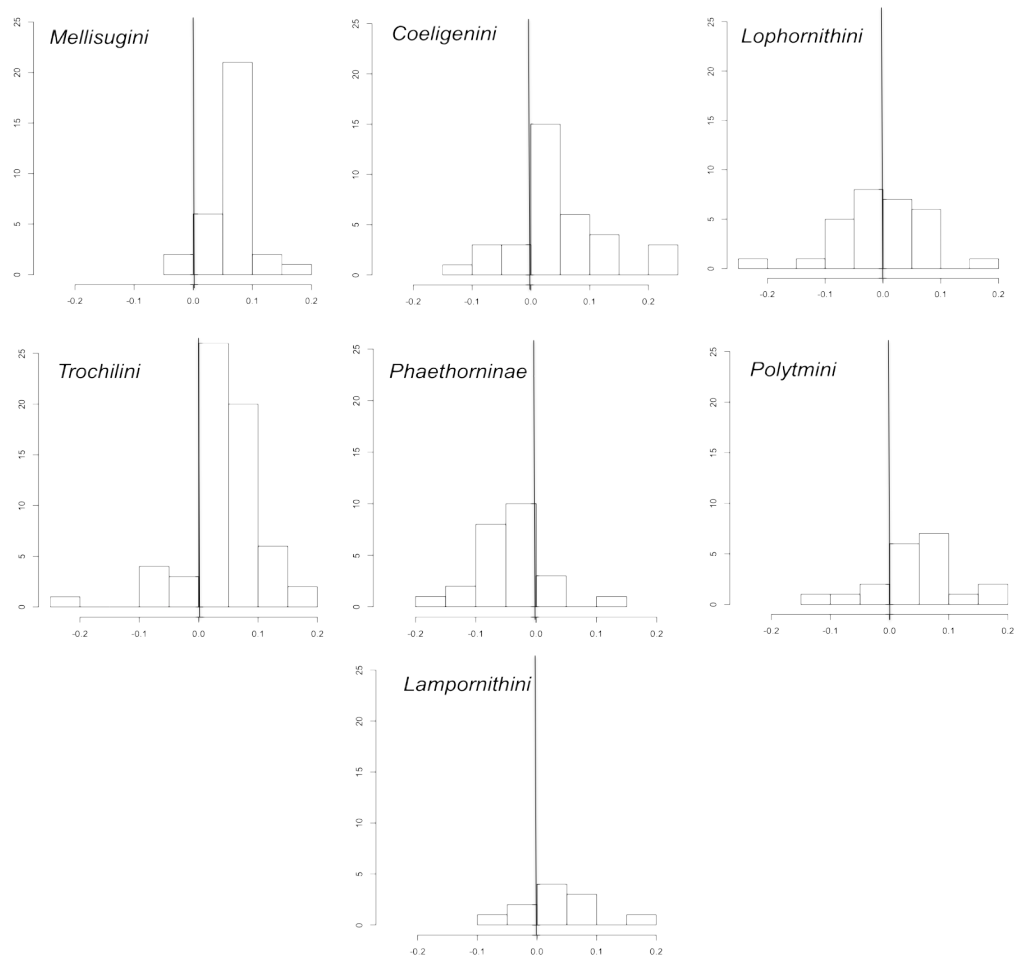


Figure 2. Histograms displaying the variation of sexual size dimorphism within each clade of hummingbirds. Frequency is on the Y-axis and bill size dimorphism (expressed as the Lovich-Gibbons ratio) is on the X-axis. Those to the left of 0 are male-biased.

Patterns of Sexual Shape Dimorphism.—Using a factorial MANOVA, we found that all factors (species, sex, species \times sex) explained significant proportions of variation in bill shape (2A). The significant interaction term implied that patterns of sexual shape dimorphism differed among species, and when this was examined separately in each

species, we found that only three of 32 species displayed significant shape dimorphism:

Archilochus colubris, *Selasphorus scintilla*, and *Mellisuga minima* (Table 2B).

Table 2. A) MANOVA analysis of bill shape (Kendall's tangent space coordinates) for 32 species in the *Mellisugini* clade. B) Results of permutational-MANOVA for those species displaying significant sexual shape dimorphism (*A. colubris*, *S. scintilla* and *M. minima*). Significant effects shown in bold.

A: Effects	Pillai's trace	Approx. F	df	P
Species	208.95	160.167	35, 1167	<0.001
Sex	470.97	361.009	1085, 37107	<0.001
Species*Sex	2.24	1.715	1085, 37107	<0.001

B: Species	Mean squares	Approx. F	P
<i>Archilochus colubris</i>	0.0137	2.944	0.04
<i>Selasphorus scintilla</i>	0.0146	3.005	0.032
<i>Mellisuga minima</i>	0.0208	4.965	0.006

Thus, in stark contrast to patterns of size dimorphism, and in contrast to patterns of shape dimorphism observed in other hummingbird lineages, very little shape dimorphism is exhibited in the *Mellisugini* lineage. When patterns of sexual shape dimorphism were compared among these three species, *M. minima* exhibited a significantly greater degree of shape dimorphism (Euclidean distance) than the other two species ($D_{M\ minima}=0.054$, $D_{A\ colubris}=0.026$, $D_{S\ scintilla}=0.041$, $P=0.001$), whereas *A. colubris* and *S. scintilla* did not differ in the amount of shape dimorphism displayed ($P=0.19$). Further, the direction of

shape dimorphism in morphospace also differed between *M. minima* and both *A. colubris* and *S. scintilla*, ($P=0.001$, Fig 3). Thin-plate spline deformation grids revealed that in these species, females have longer, more curved bills at both the tip and main body of the bill relative to the mean, while males have straighter and shorter bills and *M. minima* has the largest magnitude of sexual shape dimorphism (Fig. 3). Thus, the significant sexual shape dimorphism in these species can be generally characterized as females having more curved bills, while males have relatively straighter bills.

Discussion

For centuries, evolutionary biologists have been interested in the phenotypic differences between the sexes and the effect that these patterns have on organismal diversity. Many studies have identified patterns of sexual size and sexual shape dimorphism in hummingbird bill morphology, particularly in species from tropical lineages. However, none have used a phylogenetic context to study sexual shape dimorphism within an entire hummingbird lineage, nor compared patterns of sexual size dimorphism across all hummingbird clades in a phylogenetic context. Based on existing studies of sexual dimorphism in other species, it is generally assumed that the presence of sexual size and sexual shape dimorphism will be found throughout all hummingbird clades. However, while this appears to be the case for sexual size dimorphism of hummingbird bills, our results show that shape dimorphism in the *Mellisugini* lineage does not follow this general pattern.

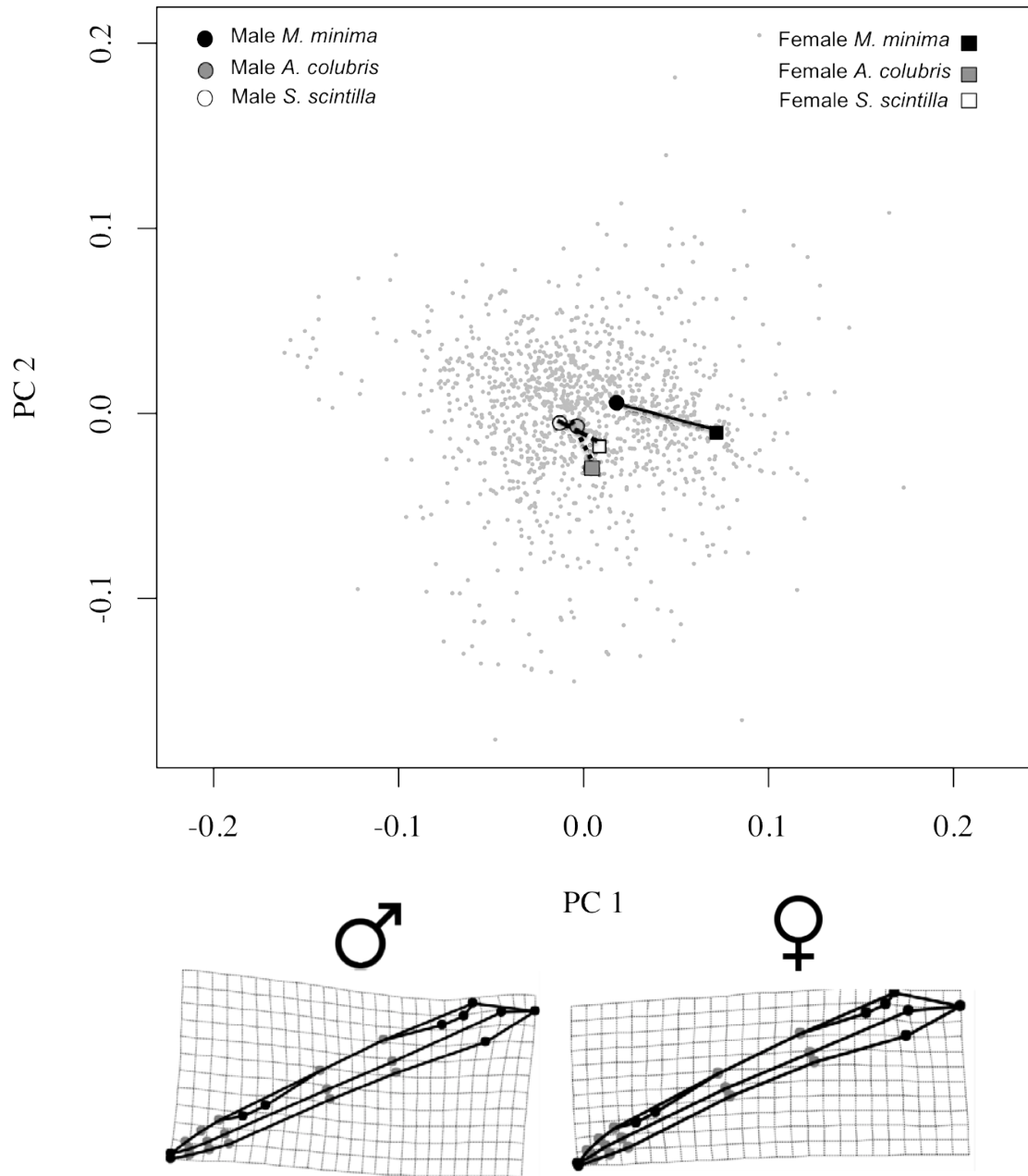


Figure 3. Principal components plot of bill shape data based on Kendall's tangent space coordinates. Here, all individuals are shown, as well as the male and female means for the three species displaying significant sexual shape dimorphism (*A. colubris*, *S. scintilla* and *M. minima*). Lines connecting symbols represent the magnitude of sexual shape dimorphism in each of the 3 species. Thin-plate spline deformation grids of the average female and male bill shape relative to the mean are also presented. The splines are the average female and average male relative to the mean and have been scaled to a factor of 2 to enhance biological interpretation.

Using bill size data for 219 species of Trochilidae, we found that the majority of the species in the *Mellisugini* clade exhibited sexual size dimorphism in similar magnitudes as was observed in other groups (Bleiweiss 1999, Colwell 2000, Temeles et al. 2010). Our findings also revealed that the *Mellisugini* lineage is decidedly female-biased in bill sexual size dimorphism, with 92% of the species examined displaying larger bills in females as compared to males. Thus, the patterns of sexual size dimorphism in *Mellisugini* are concordant with what is observed in other lineages within *Trochilidae* except *Phaethornithinae*, which is male-biased (Fig 2; also Bleiweiss 1999; Temeles et al. 2010).

The presence and magnitude of sexual size dimorphism observed here may be the consequence of differential selective forces that result from competition for ecological resources. For example, Bleiweiss (1999) hypothesized that feeding ecology may influence the evolution of male- or female-biased sexual size dimorphism in hummingbirds. In hummingbirds, a longer bill allows feeding from a wide variety of resources whereas a short bill is more efficient in a patch of the same flower. In species where the males are the dominant sex and defend territories, females tend to have longer bills, allowing them to feed from more dispersed resources, whereas males tend to have shorter bills, increasing foraging efficiency in small territories (Bleiweiss 1999). By contrast, in species with lekking mating systems, males no longer hold territories, and must instead compete with other hummingbirds. In this case, the male bill tends to be longer to allow them to feed from a wider variety of flowers due to competitive forces, whereas females feed from small patches outside the lekking grounds and have smaller

bills better suited to feeding in small patches. Thus in both cases, there is a direct relationship between sexual social interactions, foraging, and bill morphology (see Bleiweiss 1999). Our results are concordant with Bleiweiss' hypothesis, as species in the *Mellisugini* clade do not lek, and the majority display female-biased sexual size dimorphism. By contrast, species in the *Phaethornithinae* clade exhibit primarily male-biased sexual size dimorphism of bill morphology, and the majority of these species indeed have lekking behavior. It is therefore reasonable to hypothesize that the presence of female-biased sexual size dimorphism in the *Mellisugini* clade is a reflection of the non-lekking and male territorial behavior in these species, which subsequently affects differential foraging between males and females of these species.

The most surprising result of our study is that in stark contrast to other *Trochilidae* lineages, only three species (*A. colubris*, *S. scintilla* and *M. minima*) in the *Mellisugini* exhibited significant sexual shape dimorphism in bill morphology. *Trochilidae* are generally considered to be dimorphic in both bill size and bill shape, as the majority of species in some lineages display differences in bill curvature (Stiles 1995, Temeles et al. 2010). Further, individual species in many other groups, such as *E. jugularis* in the *Polytmini* clade (Temeles et al. 2005) and *Oreotrochilus estella* in the *Lophornithini* clade (Bleiweiss 1999) have also been shown to exhibit significant sexual dimorphism of both bill size and bill curvature. Together, these patterns have been interpreted as evidence that hummingbirds generally display sexual shape dimorphism in their bill morphology. However, in contrast to this general pattern, we found that nearly

all species in the *Mellisugini* clade (29 of 32 species examined) displayed no significant dimorphism in bill shape.

One possible explanation for these patterns is that hummingbird species in species-poor environments may have increased intraspecific competition, as the lack of interspecific competitors would allow the sexes to utilize distinct niches that would otherwise be occupied by congeners. Indeed, this scenario would facilitate niche separation between the sexes, which would provide possible divergent selection on bill morphology between the sexes. While this hypothesis may hold true for *A. colubris*, which breeds allopatric with respect to other hummingbird species, neither *S. scintilla* nor *M. minima* live in species-depauperate communities. Therefore, this hypothesis alone is insufficient to explain the few instances of sexual shape dimorphism displayed in *Mellisugini*. Interestingly however, *S. scintilla* is the smallest hummingbird in its range (Wood 1983) and *M. minima* is the second smallest hummingbird species in *Trochilidae* (Bird 2004). Thus, niche segregation may still be a major mechanism driving the evolution of sexual dimorphism in these taxa, as these tiny hummingbird species may not have selective pressures because larger species are not able to feed from the resources the smallest hummingbirds can feed from.

Finally, it is of interest to examine the observed patterns in the *Mellisugini* in light of their phylogenetic placement within the *Trochilidae*. Phylogenetically, *Mellisugini* are a recently diverged lineage nested deeply within *Trochilidae* (Fig. 4), and are part of a radiation that includes the evolution of several species of neotropical migrants with nearly all species within this lineage displaying little to no sexual shape dimorphism in

their bills. By contrast, lineages more basal to the *Mellisugini* display strong patterns of bill shape dimorphism, as well as bill size dimorphism. Therefore, the available data suggest the hypothesis that both bill size dimorphism and bill shape dimorphism arose early in the diversification of *Trochilidae*, and that the lack of sexual shape dimorphism presently displayed in the *Mellisugini* lineage is a derived trait.

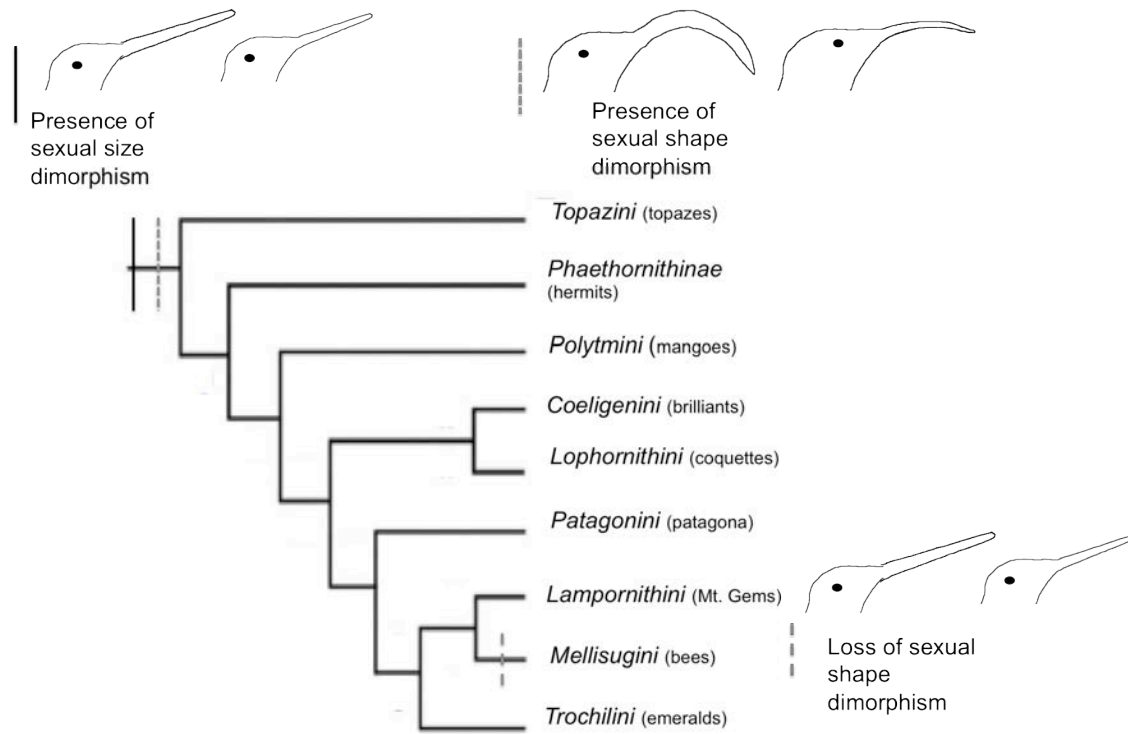


Figure 4. Current phylogeny of *Trochilidae* with emphasis on the *Mellisugini* clade. Representation of magnitude of sexual size dimorphism (SSD) and sexual shape dimorphism (SShD), stars denote significant sexual dimorphism (SD). Magnitude of SShD has been enhanced by 10 to facilitate visualization.

Taken together, the evolutionary changes in patterns of sexual size and shape dimorphism observed in *Mellisugini* suggest that the trends of sexual dimorphism in the *Trochilidae* are far more varied than was previously believed. It is possible that a combination of environmental and evolutionary factors leads to these patterns of sexual dimorphism, and further analyses examining phenotypic and environmental variation in light of phylogenetic history may reveal further insight into the underlying mechanisms driving the evolution of sexual dimorphism in the bills of hummingbirds.

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Appendix A

Number of males and females in each species of the 32 hummingbirds in the Mellisugini clade with the sexual size dimorphism ratio (negative numbers indicate male-biased SSD) and magnitude of sexual shape dimorphism.

SPECIES	Females	Males	SSD RATIO	SShD Magnitude
<i>Archilochus alexandri</i>	155	124	0.038	0.009
<i>Archilochus colubris</i>	35	42	0.119	0.026
<i>Atthis heliosa</i>	13	21	0.086	0.018
<i>Calliphlox amethystina</i>	11	14	0.031	0.017
<i>Calliphlox bryantae</i>	12	13	0.053	0.016
<i>Calliphlox evelynae</i>	13	15	-0.008	0.021
<i>Calliphlox mitchellii</i>	8	30	0.069	0.032
<i>Calothorax lucifer</i>	14	16	0.032	0.017
<i>Calothorax pulcher</i>	8	20	0.123	0.036
<i>Calypte anna</i>	13	18	0.086	0.035
<i>Calypte costae</i>	15	13	0.051	0.015
<i>Chaetocercus astreans</i>	12	12	0.082	0.022
<i>Chaetocercus bombus</i>	7	9	0.064	0.048
<i>Chaetocercus heliodor</i>	15	16	0.074	0.018
<i>Chaetocercus jourdanii</i>	15	13	0.067	0.036

Appendix A <i>(continued)</i> SPECIES	Females	Males	SSD RATIO	SShD Magnitude
<i>Chaetocercus mulsanti</i>	17	17	0.083	0.022
<i>Doricha eliza</i>	6	9	0.041	0.026
<i>Doricha enicura</i>	5	18	0.084	0.019
<i>Mellisuga helenae</i>	17	15	0.093	0.018
<i>Mellisuga minima</i>	14	13	0.091	0.058
<i>Microstilbon burmeisteri</i>	44	64	0.047	0.019
<i>Myrmia micrura</i>	8	12	0.088	0.023
<i>Myrtis fanny</i>	8	21	-0.013	0.019
<i>Rhodopis vesper</i>	10	22	0.080	0.023
<i>Selasphorus flammula</i>	20	18	0.062	0.025
<i>Selasphorus platycercus</i>	15	12	0.074	0.016
<i>Selasphorus rufus</i>	13	15	0.190	0.025
<i>Selasphorus sasin</i>	23	26	0.005	0.019
<i>Selasphorus scintilla</i>	12	13	0.059	0.044
<i>Stellula calliope</i>	15	21	0.056	0.030
<i>Thaumastura cora</i>	17	57	0.060	0.037
<i>Tilmatura dupontii</i>	14	13	0.057	0.027

Appendix B

Species sexual size dimorphism ratios from literature (negative numbers indicate male-biased SSD):

SPECIES	SSD Ratio
<i>Abeillia abeillei</i>	0.085
<i>Adelomyia melanogenys</i>	-0.065
<i>Aglaeactis castelnaudii</i>	0.016
<i>Aglaeactis pamela</i>	0.136
<i>Agliocercus kingi</i>	0.078
<i>Amazilia amabilis</i>	0.043
<i>Amazilia amazilia</i>	0.101
<i>Amazilia beryllina</i>	0.096
<i>Amazilia boucardi</i>	0.041
<i>Amazilia candida</i>	0.075
<i>Amazilia cyanocephala</i>	0.050
<i>Amazilia cyanura</i>	0.024
<i>Amazilia decora</i>	0.043
<i>Amazilia edward</i>	0.023
<i>Amazilia fimbriata</i>	0.166
<i>Amazilia franciae</i>	0.055
<i>Amazilia lactea</i>	0.059

Appendix B (continued) SPECIES	SSD Ratio
<i>Amazilia rosenbergi</i>	-0.029
<i>Amazilia rutila</i>	-0.206
<i>Amazilia saucerrottei</i>	0.082
<i>Amazilia tobaci</i>	-0.076
<i>Amazilia tzacatl</i>	0.005
<i>Amazilia violiceps</i>	0.008
<i>Amazilia viridicauda</i>	0.117
<i>Amazilia viridifrons</i>	0.149
<i>Amazilia yucatanensis</i>	0.034
<i>Androdon aequatorialis</i>	0.017
<i>Anthracothorax dominicus</i>	0.054
<i>Anthracothorax mango</i>	0.030
<i>Anthracothorax nigricollis</i>	0.036
<i>Anthracothorax prevostii</i>	0.062
<i>Anthracothorax viridis</i>	0.070
<i>Boissonneaua flavescens</i>	-0.104
<i>Boissonneaua mathewsii</i>	0.091
<i>Campylopterus curvipennis</i>	0.042
<i>Campylopterus excellens</i>	-0.063
<i>Campylopterus falcatus</i>	0.102

Appendix B (continued) SPECIES	SSD Ratio
<i>Campylopterus largipennis</i>	0.070
<i>Campylopterus villavicencio</i>	0.070
<i>Chalcostigma herrani</i>	0.007
<i>Chalcostigma ruficeps</i>	-0.037
<i>Chalcostigma stanleyi</i>	-0.112
<i>Chalybura buffonii</i>	-0.012
<i>Chalybura urochrysia</i>	0.010
<i>Chlorostilbon aureoventris</i>	-0.028
<i>Chlorostilbon auriceps</i>	0.076
<i>Chlorostilbon canivetii</i>	0.070
<i>Chlorostilbon gibsoni</i>	0.049
<i>Chlorostilbon maugaeus</i>	0.088
<i>Chlorostilbon melanorhyncus</i>	0.040
<i>Chlorostilbon mellisugus</i>	-0.073
<i>Chlorostilbon ricordii</i>	0.058
<i>Chlorostilbon swainsonii</i>	0.071
<i>Chrysolampis mosquitus</i>	0.080
<i>Chrysuronia oenone</i>	0.095
<i>Clytolaema rubricauda</i>	0.025
<i>Coeligena bonapartei</i>	0.225

Appendix B (continued) SPECIES	SSD Ratio
<i>Coeligena coeligena</i>	0.100
<i>Coeligena helianthea</i>	0.106
<i>Coeligena iris</i>	0.121
<i>Coeligena lutetia</i>	0.047
<i>Coeligena phalerata</i>	0.202
<i>Coeligena torquata</i>	0.055
<i>Coeligena violifer</i>	-0.065
<i>Coeligena wilsoni</i>	0.068
<i>Colibri coruscans</i>	-0.042
<i>Colibri delphinae</i>	-0.129
<i>Colibri thalassinus</i>	0.093
<i>Cyanthus latirostris</i>	0.069
<i>Cynanthus sordidus</i>	0.025
<i>Damophila julie</i>	0.077
<i>Discosura conversii</i>	0.027
<i>Doryfera johannae</i>	0.052
<i>Doryfera ludovicae</i>	0.050
<i>Elvira chionura</i>	0.013
<i>Elvira cupreiceps</i>	0.035
<i>Ensifera ensifera</i>	-0.030

Appendix B (continued) SPECIES	SSD Ratio
<i>Eriocnemis alinae</i>	0.014
<i>Eriocnemis cupreiventris</i>	0.026
<i>Eriocnemis derbyi</i>	0.048
<i>Eriocnemis luciana</i>	-0.003
<i>Eriocnemis mosquera</i>	0.012
<i>Eriocnemis nigrivestis</i>	0.037
<i>Eriocnemis vestitus</i>	0.079
<i>Eugenes fulgens</i>	0.050
<i>Eulampis holosericeus</i>	0.152
<i>Eulampis jugularis</i>	0.180
<i>Eupherusa cyanophrys</i>	0.108
<i>Eupherusa eximia</i>	0.012
<i>Eutoxeres aquila</i>	-0.040
<i>Eutoxeres condamini</i>	0.010
<i>Florisuga mellivora</i>	0.010
<i>Glaucis aenea</i>	-0.020
<i>Glaucis hirsutus</i>	-0.003
<i>Haplophaedia aureliae</i>	0.016
<i>Heliangelus amethysticollis</i>	0.063
<i>Heliangelus exortis</i>	-0.060

Appendix B (continued) SPECIES	SSD Ratio
<i>Helianthus viola</i>	-0.074
<i>Heliodoxa aurescens</i>	0.030
<i>Heliodoxa branickii</i>	0.006
<i>Heliodoxa gularis</i>	-0.065
<i>Heliodoxa imperatrix</i>	0.040
<i>Heliodoxa jacula</i>	0.047
<i>Heliodoxa leadbeateri</i>	0.069
<i>Heliodoxa rubinoides</i>	0.045
<i>Heliodoxa schreibersii</i>	-0.027
<i>Heliodoxa xanthogonys</i>	0.208
<i>Heliomaster constantii</i>	0.009
<i>Heliothryx aurita</i>	0.062
<i>Heliothryx barroti</i>	0.013
<i>Hylocharis chrysura</i>	0.017
<i>Hylocharis cyanus</i>	0.011
<i>Hylocharis eliciae</i>	0.042
<i>Hylocharis grayi</i>	0.033
<i>Hylocharis leucotis</i>	0.048
<i>Hylocharis xantusii</i>	0.006
<i>Klais guimeti</i>	0.015

Appendix B (continued) SPECIES	SSD Ratio
<i>Lafresnaya lafresnayi</i>	0.133
<i>Lampornis amethystinus</i>	0.035
<i>Lampornis calolaema</i>	0.074
<i>Lampornis castaneiventris</i>	0.080
<i>Lampornis cinereicauda</i>	0.057
<i>Lampornis clemenciae</i>	0.170
<i>Lampornis rhami</i>	-0.046
<i>Lampornis sybillae</i>	0.043
<i>Lampornis viridipallens</i>	-0.084
<i>Lesbia nuna</i>	0.000
<i>Lesbia victoriae</i>	-0.042
<i>Leucippus baeri</i>	-0.078
<i>Leucippus chlorocercus</i>	0.000
<i>Leucippus hypostictus</i>	0.138
<i>Leucippus taczanowskii</i>	0.051
<i>Loddigesia mirabilis</i>	0.103
<i>Lophornis adorabilis</i>	0.035
<i>Lophornis delattrei</i>	0.073
<i>Lophornis ornatus</i>	0.031
<i>Metallura aeneocauda</i>	0.058

Appendix B (continued) SPECIES	SSD Ratio
<i>Metallura eupogon</i>	-0.080
<i>Metallura iracunda</i>	-0.004
<i>Metallura odomae</i>	-0.032
<i>Metallura phoebe</i>	-0.213
<i>Metallura tyrianthina</i>	0.047
<i>Metallura williami</i>	0.153
<i>Oreonympha nobilis</i>	-0.004
<i>Oreotrochilus estella</i>	-0.031
<i>Orthrorhyncus cristatus</i>	0.185
<i>Panterpe insignis</i>	-0.013
<i>Phaeochroa cuvieri</i>	0.033
<i>Phaethornis anthophilus</i>	-0.100
<i>Phaethornis astrimentalis</i>	-0.008
<i>Phaethornis augusti</i>	-0.005
<i>Phaethornis bourcieri</i>	-0.040
<i>Phaethornis griseogularis</i>	0.150
<i>Phaethornis guy</i>	-0.080
<i>Phaethornis hispidus</i>	-0.070
<i>Phaethornis koepckae</i>	-0.090
<i>Phaethornis longirostris</i>	-0.060

Appendix B (continued) SPECIES	SSD Ratio
<i>Phaethornis longuemareus</i>	0.020
<i>Phaethornis malaris</i>	-0.050
<i>Phaethornis philippii</i>	-0.090
<i>Phaethornis pretrei</i>	-0.053
<i>Phaethornis ruber</i>	-0.006
<i>Phaethornis striigularis</i>	-0.079
<i>Phaethornis subochraceus</i>	-0.163
<i>Phaethornis superciliosus</i>	-0.029
<i>Phaethornis syrmatophorus</i>	-0.030
<i>Phaethornis yaruqui</i>	-0.120
<i>Phlogophilus harterti</i>	0.006
<i>Polyonimus caroli</i>	-0.025
<i>Polytmus guainumbi</i>	0.004
<i>Polytmus theresiae</i>	-0.008
<i>Popelairia langsdorffi</i>	-0.063
<i>Pterophanes cyanopterus</i>	0.007
<i>Ramphomicron microrhynchum</i>	-0.014
<i>Sappho sparganura</i>	0.055
<i>Schistes geoffroyi</i>	-0.053
<i>Sephanoides fernandensis</i>	0.006

Appendix B (continued) SPECIES	SSD Ratio
<i>Sephanoides sephanoides</i>	0.081
<i>Thalurania colombica</i>	0.018
<i>Thalurania furcata</i>	0.076
<i>Thalurania glaucopis</i>	0.072
<i>Threnetes leucurus</i>	0.006
<i>Threnetes ruckeri</i>	-0.020
<i>Topaza pella</i>	0.020
<i>Topaza pyra</i>	-0.055
<i>Trochilus polytmus</i>	0.030
<i>Urosticte benjamani</i>	-0.066

Appendix C

Specimens examined to quantify morphological variation:

We examined the left lateral side of bills from collections at Carnegie Museum of Natural History (CM), Cornell University Museum of Vertebrates (CUMV), Delaware Museum of Natural History (DMNH), Field Museum of Natural History (FMNH), Los Angeles County Museum (LACM), Museum of Comparative Zoology (MCZ), Museum of Southwestern Biology (MSB), Museum of Vertebrate Zoology (MVZ), San Diego Natural History Museum (SDNHM), University of Michigan Museum of Zoology (UMMZ), National Museum of Natural History (NMNH), Western Foundation of Vertebrate Zoology (WFVZ), and Yale Peabody Museum (YPM). Specific specimens, listed by institution, include the following:

AMNH: 100678, 109537, 117704, 124278, 124279, 124280, 124281, 13002, 131195, 145839, 151434, 151437, 151458, 151459, 170352, 171148, 171149, 171157, 179065, 181241, 182361, 229094, 234478, 235492, 235680, 235859, 305591, 305592, 229094, 234478, 235492, 235680, 235859, 305591, 305592, 326286, 326287, 326290, 326292, 326293, 326295, 326296, 361892, 361894, 337924, 61896, 361897, 361898, 37784, 37893, 37894, 37896, 37903, 37905, 37911, 37938, 37941, 37945, 37947, 37949, 37950, 37952, 37953, 37957, 37981, 38004, 38715, 389741, 394200, 394202, 394204, 394206, 394208, 394215, 394217, 394218, 394219, 394220, 394222, 437741, 46310, 46608, 46631, 46634, 46636, 46637, 46655, 46659, 46713, 46736, 484400, 484402, 484403, 484407, 484408, 484409, 484525, 484529, 484547, 484550, 484551, 484553, 484554,

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YPM: 6442, 99650

CHAPTER 5
MACROEVOLUTIONARY PATTERNS OF SEXUAL SIZE AND SHAPE
DIMORPHISM IN HUMMINGBIRDS

Chelsea Berns

Abstract

Many evolutionary predictions have been posited to understand macroevolutionary patterns of diversity, one of which is that rates of evolution will be related to these patterns such that one could predict changes in phenotypic evolution from rates of morphological evolution. However, while rates of evolution are frequently examined for measured traits such as body size or limb proportions, other species-level traits, such as the degree of sexual dimorphism, remain under-examined. Here we quantify sexual size and sexual shape dimorphism from the bills of 269 species and use a recent time-dated molecular phylogeny to examine rates of phenotypic evolution in hummingbirds. We find that size and shape dimorphism do not associate with one another at a macroevolutionary level, and are thus evolutionarily decoupled. Furthermore, size dimorphism is more common and evolutionarily labile than is shape dimorphism. Intriguingly, we also find that the rate of sexual shape dimorphism, species richness, and rate of species diversification are all positively correlated, implying that these are predictors of species richness. A number of causal mechanisms may drive these relationships. We hypothesize that ecological opportunity, driven by resource utilization and the exploitation of new niches, is a possible driving mechanism of the observed macroevolutionary patterns of hummingbird diversity.

Introduction

Patterns of biodiversity provided the framework for Darwin's theory of descent with modification, which laid the foundation for the field of evolutionary biology (Darwin, 1859). Understanding macroevolutionary patterns of biodiversity has remained of central interest to evolutionary biologists over the last century. Indeed, quantification of patterns can test predictions that stem from hypotheses about mechanisms underlying diversity. In particular, it has long been suggested that differences in rates of morphological evolution between lineages may be a major factor underlying patterns of biodiversity and impacting phenotypic trends within and across lineages. Simpson (1944, 1953) was amongst the first to quantify morphological rates of evolution and suggested that diversity arose from simultaneous divergences of many lines of species stemming from a single ancestral adaptive type. Similarly, three main causal explanations for these patterns of macroevolution were identified (patterns of evolution above the species level: Futuyma, 2005), specifically speciation, phyletic mode, and quantum evolution. When speciation explains macroevolution, evolutionary rates would be erratic with no general trend. 'Phyletic' mode describes an evolutionary pattern when all species in a lineage are changed by sustained selection, which then results in a moderate rate of change, while 'quantum evolution' results in drastic shifts in adaptive zones and rapid rates of evolutionary change (Simpson, 1944, 1953).

An alternative model to those of Simpson ("punctuated equilibrium": *sensu* Gould and Eldredge (1977) posits that over macroevolutionary timescales, there may be long periods with little change (stasis) that are interrupted by periods of higher rates of change

(punctuation events). From this alternative model, several testable predictions then arise. For example, if species mostly exhibit stasis, then rates of morphological change in a clade may be positively correlated with rates of speciation, as the two are temporally restricted to those time periods when phyletic and phenotypic changes co-occur. Further, trends in mean morphology among the species of a clade will not be the result of the long-term transformation of existing lineages (anagenesis), but will result from sequences of differential speciation or extinction (Damuth, 2001). Importantly, such patterns may be the result of ecological mechanisms, such as the opening of new niches, or with radical changes in environment.

Under some macroevolutionary scenarios (e.g., punctuated equilibrium), one might expect a relationship between species diversity and morphological disparity if adaptive evolution continues over long time spans. For example, Ricklefs (2004, 2006) identified a significant trend between species richness and morphological diversity across sub-clades of passerines, and from this he concluded that selective (adaptive) processes were responsible for the observed patterns. One can then test whether isolated populations, rapid changes in the composition of the gene pool, or community structure occurring gradually over time generated the observed patterns. Interestingly, a re-examination of these data demonstrated that the pattern was equally well explained by simply the age of the sub-clades, suggesting that neutral, and not adaptive processes were a viable explanation for the trends seen across these species (Purvis, 2004).

Schluter (2000) summarized the empirical macroevolutionary literature and found that species richness was positively correlated morphological variation in many clades,

including: East African cichlid fishes, Hawaiian silverswords, and “Darwin’s finches”, amongst others. These patterns suggest that the evolution of adaptive phenotypes can be common, and is thus a major factor driving divergence and speciation across evolutionary timescales. Under this scenario, new species develop the ability to utilize empty niche space due to adaptive morphological divergence and resource specialization (‘ecological theory of adaptive radiation’). Another prediction consistent with the hypothesis of adaptive radiations is that increased species richness will relate to higher rates of morphological evolution (Foote, 1997; Harmon et al., 2003; Ricklefs, 2004, 2006). Harmon *et. al* (2003) found that in *Anolis*, taxa with steady rates of increase in species richness have increased morphological variation of limb length within clades, but that taxa with early bursts of lineage accumulation have very little within-clade variation. Ecology and the ability to fill ecological niches is a major process underlying species richness, with taxa diversifying early in their history and filling all available niche space with little opportunity for more diversification while those lineages that diversify more slowly have greater opportunity for morphological changes (but see Harmon et al., 2010 for a recent empirical counterpoint). Furthermore, greater morphological change is associated with greater diversification, leading to the evolution of phenotypic adaptation (Schluter, 2000).

When rates of phenotypic evolution are examined in a macroevolutionary context, studies typically quantify rates for single, univariate traits, such as body size (Harmon et al., 2010), proportion of limb length (Mahler et al., 2010), and aspects of body shape (Schoener, 2011). Additionally, several recent studies have quantified evolutionary rates

for multiple traits treated simultaneously in a multivariate framework (e.g., McPeck et al. 2008; Adams et al. 2009; Revell and Collar 2009; Martin and Wainwright, 2011; Adams, 2013). In such studies, each species is represented by a single value per trait (typically the mean), which is used in a comparative framework to obtain estimates of the rate of evolution of that trait in a phylogenetic context. However, evolutionary biologists are also interested in how other species-level characteristics evolve through time, and in theory, rates of evolution of these traits could be examined using an identical procedure. One such characteristic is sexual dimorphism (SD). Here, differences between the sexes, such as in coloration (Endler, 1983), behavior (Clyne and Miesenböck, 2008), vocalizations (Price, 1998) or ornamentation (Worthington et al., 2012), may be quantified for each species and used as species-level data in a phylogenetic comparative analysis. Indeed, the consequences of SD for the ecology and evolution of organisms are often profound and thus, informs our understanding of a number of mechanisms that have been proposed to explain the variable distribution of SD among taxa (Berns, 2013; Berns and Adams, 2013; Fairbairn et al., 2007; Stephens and Wiens, 2009; Worthington et al., 2012). For example, mate choice has been suggested as an important proximate mechanism of sexual selection (Darwin, 1871). Alternatively, ecological mechanisms such as competition for resources may exert distinct selective forces on the sexes, resulting in the evolution of sexual dimorphism (Eberhard, 1985). Conversely, many studies have suggested that competition for mates is at the very heart of sexual dimorphism (Bean and Cook, 2001).

In some species, sex-specific differences in size represent an ecologically

important component of sexual dimorphism (SSD; Berns and Adams, 2010; Stephens and Wiens, 2009), and numerous mechanisms may be implicated in the evolution of SSD. For example, SSD can be driven by differences in the reproductive roles of males and females (Dale et al., 2007; Ralls, 1976; Rensch, 1960), where females are larger than males where larger size increases number and quality of offspring. Conversely, males may be larger than females when larger males can out-compete smaller males to more successfully obtain mates (Benson and Basolo, 2006). In addition to size dimorphism, differences in the shape of ecologically-relevant anatomical traits may also be displayed between the sexes (termed sexual shape dimorphism: SShD). Surprisingly, while shape contributes to various functions such as feeding, parental care and mating, SShD remains understudied (Gidaszewski et al., 2009; Hedrick and Temeles, 1989; Lande and Arnold, 1985).

Hummingbirds (Trochilidae) provide an ideal system to study the evolution of sexual size and shape dimorphism, as many species display sexual dimorphism in their bill morphology (Berns and Adams, 2010; Berns and Adams, 2013). In hummingbirds, differences in bill morphology are tightly linked with feeding efficiency and foraging preferences between species (Darwin, 1871; Gould, 1861), as well as between the sexes (Bleiweiss, 1999a; Cotton, 1998; González-Gómez and Estades, 2008; Stiles, 1995a). For instance, males and females of *Eulampis jugularis* have different bill sizes and shapes due to the different floral morphology of the Heliconia flowers they feed from (*H. bihai* and *H. caribaea*; Temeles and Kress, 2003; Temeles et al., 2000). In another well-studied clade of hummingbirds (the Hermit clade: *Phaethornithinae*), the majority of

species exhibit sexual dimorphism in bill shape, which is also tied to different floral morphology (Temeles et al., 2010). In contrast to other species however, some hummingbird clades (e.g., the Bees clade: *Mellisugini*) exhibit very little bill shape dimorphism across taxa (Berns and Adams, 2013). Differences in community composition has been proposed to explain these patterns, as hummingbirds in species-poor environments have decreased intraspecific competition, allowing the sexes to utilize distinct niches that would otherwise be occupied by conspecifics (Berns and Adams, 2010). Taken together, we expect that the majority, but not all, of hummingbird species will display sexual dimorphism in their bill morphology.

In this study we quantify the size and shape dimorphism in hummingbird bills across species and use these species-level traits to address the following evolutionary questions: 1) What are the patterns of sexual size and shape dimorphism in hummingbird bill morphology? 2) Are sexual size and shape dimorphism correlated such that the rates of evolution of size and shape dimorphism significantly influence one another? 3) Across sub-clades of hummingbirds are patterns of phenotypic diversity associated with: rates of evolution of sexual dimorphism, clade age, percent of species with bill dimorphism, species richness, magnitude of dimorphism, and/or species diversification? Based on previous studies, we expect that patterns such as increased species richness and clade age should relate to higher amounts of phenotypic disparity and rates of morphological evolution. We further predict that as these clade-level descriptors increase, so will the percent of species in each clade that exhibit sexual size/shape dimorphism, as will the rate of species diversification. Finally, we predict that clade age

will be significantly associated with variation due to the accumulation of morphological diversity over time. Here, we quantify bill morphology from 269 species of hummingbirds to address these questions in order to understand patterns of the evolution of sexual dimorphism and ultimately, the evolution of biodiversity.

Methods

Specimen information.- We measured 6,794 hummingbirds representing 269 of the 338 currently recognized hummingbird species (Birdlife.org, 2012). From these, we had sufficient data to analyze 7 of the 8 clades in Trochilidae (we excluded *Topaza* due to small sample size; see Figure 1). Specimens were obtained from 14 museum collections (see Appendix) and were adults of known sexes. A roughly equal proportion of males and females were used in this study (total: 3,654 males, 3,140 females; see Appendix).

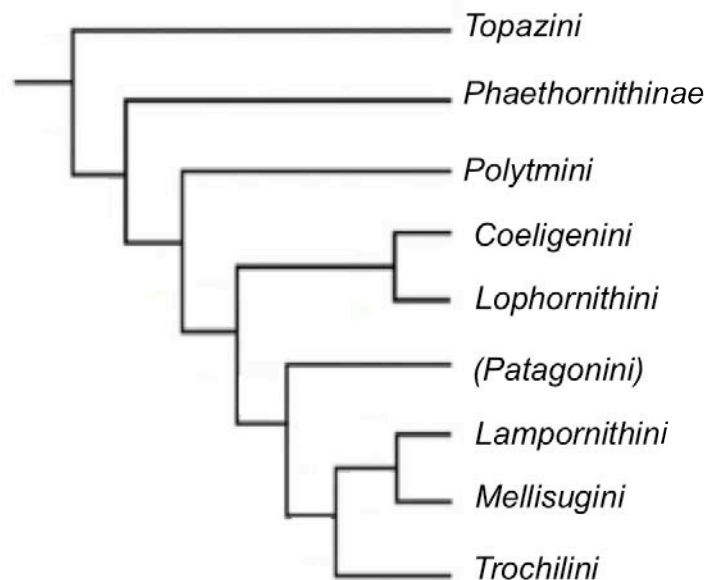


Figure 1. Current phylogeny backbone of *Trochilidae*, based on McGuire et al., 2007

Morphometrics.- Estimates of bill size and bill shape were quantified from digital images of each specimen. First we obtained digital images of the left-lateral side of the head and bill of each specimen using a Nikon DXM-1200 digital camera mounted on a Nikon SMZ 1500 stereomicroscope (a Nikon D-90 was used to collect images obtained at museums, as the stereomicroscope system was not easily transportable). Birds were placed in the same position on a modeled clay surface to minimize distortion, and each photograph included a ruler to account for size in the analyses. From these images, we collected bill size linear measurements as the exposed culmen length for each individual (Fig. 1A; e.g., Bleiweiss, 1999b; Colwell, 2000). We also obtained shape variables from each image using landmark-based geometric morphometric methods (Adams et al., 2004; Adams et al., 2013; Rohlf and Marcus, 1993). These methods provide a rigorous quantification of shape after non-shape variation (i.e., differences in position, orientation and scale) has been mathematically removed.

For the latter approach, we digitized the positions of 10 biologically homologous landmarks on the images of each specimen and 15 sliding semi-landmarks to capture all detailed bill curvature. Once digitized using TPSDIG2 (Rohlf, 2010a), these 25 landmarks were combined to form 50 coordinates that were then used to represent bill shape (Fig. 2B). We removed non-shape variation using a generalized Procrustes analysis, which superimposes the landmarks on each specimen using least-squares to translate them to the origin, re-size them to the unit centroid size, and finally rotate them to minimize the sums-of-squares differences of the landmark coordinates from all specimens to the average form.



Figure 2. Exposed culmen (a), and landmark-based morphometrics (b), shown on a Black-chinned Hummingbird

During this analysis, the sliding semi-landmarks were mathematically slid along their tangent directions to minimize the Procrustes distances between each specimen and the average. Once orthogonally projected into a linear tangent space, the resulting aligned Procrustes shape coordinates describe each specimen and this set of shape variables are used for all further analyses of shape variation. These procedures were performed in TPSRELW (Rohlf, 2010b).

Patterns of size dimorphism.- Using linear measurements of the exposed culmen length (CL) of all specimens, we determined if there was significant sexual size dimorphism and if this varies among species using a two-factor analysis of variance

(ANOVA) with species, sex, and a species \times sex interaction. Another measure of bill size (centroid size) was highly correlated with CL for these data ($r=0.97$), and was not used in subsequent analyses. Analyses were also performed on each species separately due to large variances within the species \times sex groups. From the original two-factor model we obtained the average male and female bill size ($\overline{CL}_M, \overline{CL}_F$). From these, patterns of sexual size dimorphism for each species was summarized using the Lovich-Gibbons ratio (1992), which is found as: $(\overline{CL}_F/\overline{CL}_M - 1)$ (see Stephens and Wiens, 2009; Temeles et al., 2010), where this is multiplied by (1) in species where the female is the larger sex and (-1) where the males are larger. We then performed an ANOVA on these ratios to determine whether patterns of size dimorphism differed among clades. In addition, to account for non-independence due to shared evolutionary history in this analysis, we performed a phylogenetic ANOVA (Garland et al., 1993) where the evolutionary relationships among species were based on the current molecular phylogeny for hummingbirds (McGuire et al., 2007), as well as estimated taxonomic relationships (McGuire et al., 2009).

Patterns of shape dimorphism.- To determine which species exhibit significant sexual shape dimorphism, we used the shape variables obtained using geometric morphometrics to perform a permutational multivariate analysis of variance (MANOVA; 9,999 iterations) on each species separately, due to the large variation between species. Next we calculated multivariate vectors of shape dimorphism for each species, where these vectors connect male and female phenotypic means and the length of these vectors (Euclidean distance) is considered as a measure of the magnitude of shape dimorphism

(for details see Adams and Collyer, 2007; Adams and Collyer, 2009; Collyer and Adams, 2007). We then compared these vectors using a permutation procedure with 9,999 iterations to determine whether the degree of sexual shape dimorphism differed among species (see Berns and Adams, 2010).

Rates of evolution.- To examine rates of phenotypic evolution in a phylogenetic context, we used a recent multi-gene time-calibrated molecular phylogeny of hummingbirds (McGuire et al., 2007), which included 151 of 338 species divided into 8 clades (see Fig. 1; Birdlife.org, 2012). We first matched this with the species for which we had phenotypic data and time-calibrated the existing molecular phylogeny using divergence dates among 28 hummingbird species obtained from a least-squares analyses of DNA-DNA hybridization (Bleiweiss, 1998b). From that analysis we obtained values for 6 calibration points on the larger phylogeny that matched nodes of the existing tree and constructed a time-dated chronogram using penalized likelihood (Sanderson, 2002) in R (R Development Core Team, 2012). Next we pruned the time-calibrated phylogeny (which contained 151 species) to the set of species that co-occurred in our dataset, resulting in a time-calibrated tree for 134 species (Fig. 3). From this we estimated the rate of evolution of sexual size and shape dimorphism assuming a Brownian motion model of evolution (σ^2 : O'Meara et al., 2006).

Using this phylogeny, we first quantified the rate of bill size evolution in both males and females, and compared these rates using a recently developed likelihood method (Adams, 2013).

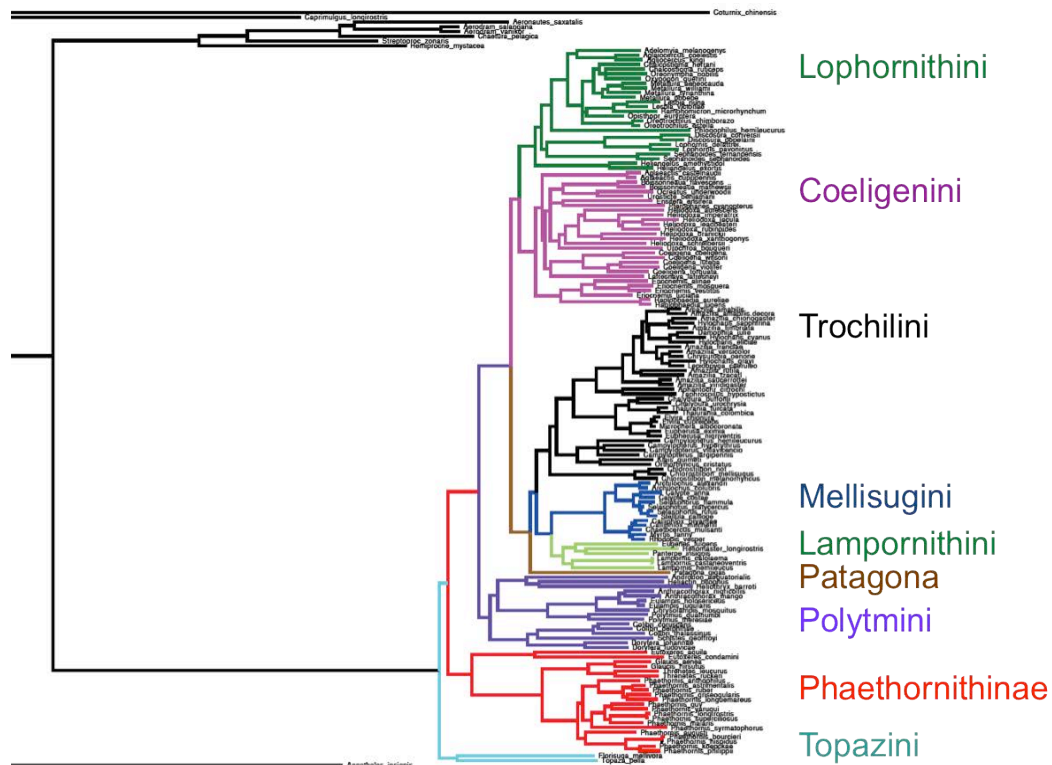


Figure 3. Division into 9 subclades based on McGuire et al., 2007, 2009

With this approach, the likelihood of a model where the bill size in males and females across species evolve at separate rates is first obtained, and is compared to the likelihood of a model where the sexes are constrained to evolve at a common rate. The two likelihoods are then evaluated statistically using likelihood ratio tests, with the trait with the largest σ^2 evolving at a faster rate (see Adams, 2013). We then used this method to compare rates of evolution between sexual size and sexual shape dimorphism and performed this rate comparison at two phylogenetic scales within Trochilidae: first across the entire phylogeny for the family, and second within each of the seven major clades as found above (*sensu* McGuire et al., 2007). For all comparisons, evolutionary rates of

sexual dimorphism were found using a Brownian motion model of evolution. To determine which clades had overlapping rates, 95% confidence intervals were estimated from the standard errors of each rate, found from the Hessian matrix of the multi-rate model (see Revell, 2013). Overlapping confidence intervals would suggest that the clades do not have statistically different rates.

Comparison of sexual size and shape dimorphism.- For each clade we tested the relationship between sexual size and sexual shape dimorphism by performing clade-level analyses using phylogenetic generalized least squares (PGLS: Martins, 1994) using the rates of size and shape dimorphism of the 7 clades as data. Specifically, to test whether patterns of size and shape dimorphism are linked we quantified the relationships between i) rates of evolution of sexual size dimorphism with magnitude of sexual shape dimorphism, ii) the rate of sexual size dimorphism and percent of species in each clade with shape dimorphism, iii) rates of evolution of shape dimorphism with magnitude of sexual size dimorphism iv) rates of evolution of shape dimorphism, and v) the percent of species in each clade with size dimorphism.

Additional clade-level descriptors.- Using the sexual size and shape measures described above, we associated changes in these characteristics with several additional clade-level descriptors. For this we estimated the following characteristics for each sub-clade: i) the percent of species with sexual dimorphism, ii) clade age, iii) species diversity and iv) species diversification rate. These descriptors were based on the full species richness for Trochilidae (331 species), which were assigned to sub-clades based on the taxonomy from McGuire et al. 2007; McGuire et al. 2009. Following Adams et al. (2009)

we used then PGLS under a Brownian motion model to examine the relationships of these clade-level descriptors with sexual size and shape dimorphism separately to evaluate the relative correlations of both traits to each parameter. All analyses performed in R (R Development Core Team, 2012).

Results

Sexual size dimorphism.- Using an ANOVA on bill size measurements, we found significant differences in the degree of sexual size dimorphism exhibited among species ($F_{\text{species} \times \text{sex}} (1,268) = 3.699, P < 0.001$). About half of the species examined (135 of 269) exhibited significant sexual size dimorphism with a large variation in magnitudes (Appendix Table 1). Overall we found no difference in the degree of size dimorphism among clades, suggesting that the entire Trochilidae lineage displays similar degrees of sexual size dimorphism among its sub-lineages ($F_{8,260} = 0.808, P = 0.595$). These patterns were robust when taking phylogenetic relatedness into account (phylogenetic ANOVA: $F_{8,126} = 0.612; P_{\text{rand}} = 0.766$).

Sexual shape dimorphism.- Using MANOVA, we found significant differences in the degree of sexual shape dimorphism exhibited among species ($F_{\text{species} \times \text{sex}} (1,268) = 2.320, P < 0.001$). We found that only 46 of the 269 species examined (17%) display significant sexual shape dimorphism of their bills. When examined by sub-lineage, as with size dimorphism, we found differences in the degree of shape dimorphism among clades, such that Trochilidae overall does not display differing degrees of sexual shape dimorphism among its sub-lineages ($F_{8,260} = 0.395, P = 0.9047$; see Appendix Table 2).

These patterns yielded biologically similar results when accounting for phylogeny (phylogenetic ANOVA: $F_{8,126} = 0.565$; $P_{\text{rand}} = 0.7831$).

Rates of evolution.- Rates of evolution of bill size between males and females did not differ phylogenetically ($\sigma^2_{\text{male}} = 0.0247$, $\sigma^2_{\text{female}} = 0.022$, $P = 0.722$), implying that the resulting size dimorphism was not driven by faster bill size evolution in one sex or the other. By contrast, rates of sexual size and sexual shape dimorphism for all of Trochilidae do differ, with sexual size dimorphism evolving at a much faster rate than sexual shape dimorphism ($\sigma^2_{\text{Size}} = 0.1730$, $\sigma^2_{\text{Shape}} = 0.0004$, $P = <0.001$). This finding implies that variation in sexual size dimorphism across species accumulates more rapidly in hummingbirds than variation of shape dimorphism.

We found that the rate of evolution of sexual size dimorphism varied greatly among clades (*Lampornithini* clade: $\sigma^2 = 0.03$; *Trochilini* clade: $\sigma^2 = 0.36$), with most clades displaying significantly different rates of evolution (Table 1A; Fig 4A). The exception to this pattern was the *Mellisugini* and *Phaethornithinae* clades, whose evolutionary rates were relatively low and displayed overlapping confidence intervals; thus showing no difference in evolutionary rates (Table 1B). When comparing rates of sexual shape dimorphism among clades, as with size dimorphism, we found a fivefold difference in rates among clades (*Mellisugini* clade: $\sigma^2 = 0.0013$; *Coeligenini* clade: $\sigma^2 = 0.0066$). Here, *Lampornithini*, *Phaethornithinae*, *Coeligenini* and *Lophornithini* displayed overlapping confidence intervals, again suggesting that these clades do not differ in their rates of evolution of shape dimorphism (see Table 1A, B; Fig. 4B).

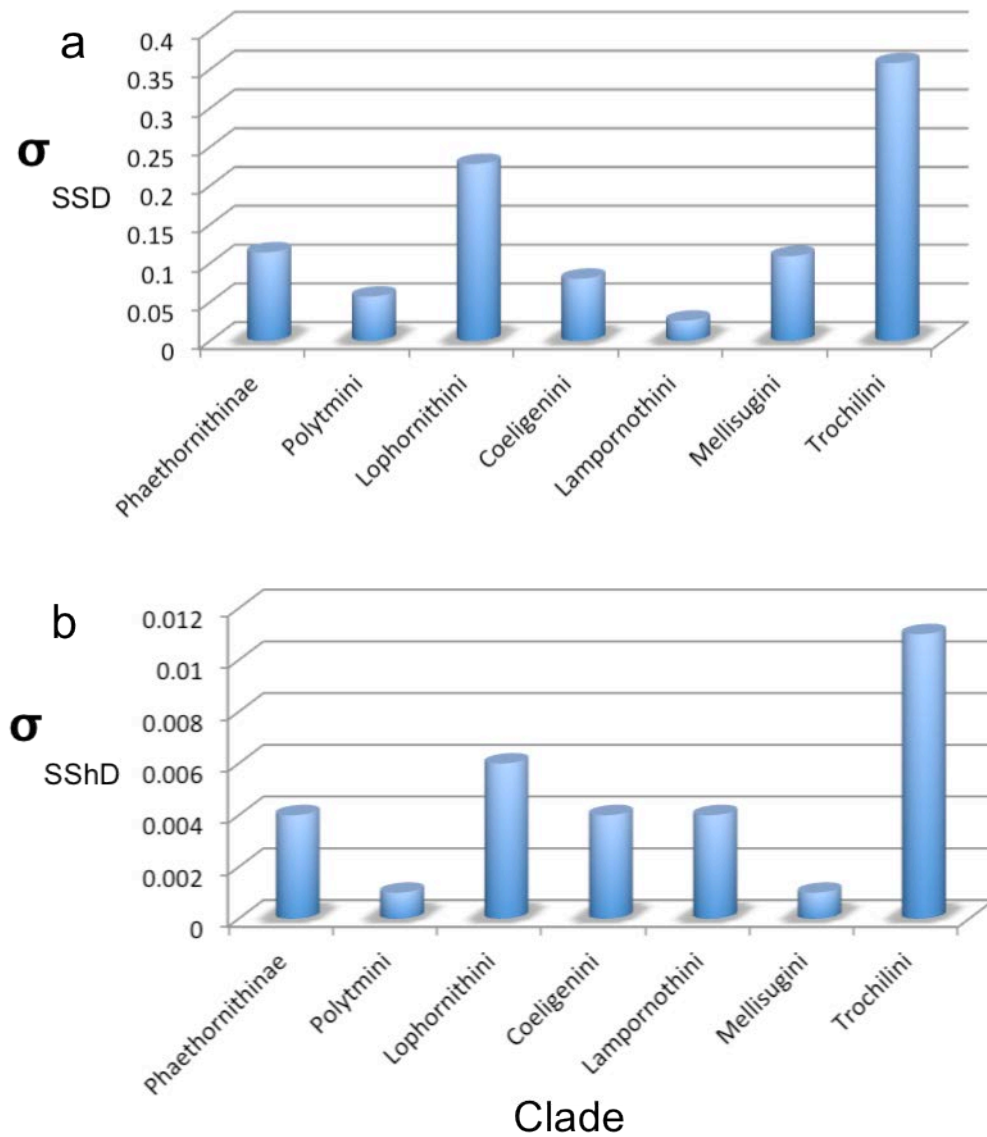


Figure 4. Sexual size dimorphism showing the two clades that overlap in confidence intervals, *Mellisugini* and *Phaethornithinae* (a) and sexual shape dimorphism (b) showing the clades that overlap in confidence intervals, *Lampornithini*, *Phaethorninae*, *Coeligenini* and *Lophornithini*.

Table 1. A) Rates of sexual size (SSD) and shape (SShD) dimorphism in each clade and B) 95% confidence intervals estimated from standard errors of each rate. Bold denotes those clades that overlap.

A. Clade	σ^2 SSD	σ^2 SShD
<i>Phaethornithinae</i>	0.1148	0.0045
<i>Polytmini</i>	0.0578	0.0018
<i>Lophornithini</i>	0.2281	0.0062
<i>Coeligenini</i>	0.0806	0.0066
<i>Lampornithini</i>	0.0266	0.0046
<i>Mellisugini</i>	0.1091	0.0013
<i>Trochilini</i>	0.3581	0.0115

B. Clade	Upper CI, SSD	Lower CI, SSD	Upper CI, SShD	Lower CI, SShD
<i>Phaethornithinae</i>	0.1210*	0.1086*	0.0048*	0.0044*
<i>Polytmini</i>	0.0611	0.0544	0.0024	0.0022
<i>Lophornithini</i>	0.2405	0.2158	0.0065*	0.0059*
<i>Coeligenini</i>	0.0842	0.0770	0.0074*	0.0068*
<i>Lampornithini</i>	0.0302	0.0231	0.0053*	0.0038*
<i>Mellisugini</i>	0.1163*	0.1020*	0.0013	0.0011
<i>Trochilini</i>	0.3734	0.3428	0.0116	0.0106

Comparison of sexual size and shape dimorphism.- Using PGLS we found that rates of evolution of sexual size and shape dimorphism were not significantly correlated

with one another across hummingbird sub-clades. Further, non-significant relationships were found between i) rates of evolution of sexual size dimorphism with magnitude of sexual shape dimorphism, ii) the rate of sexual size dimorphism and percent of species in each clade with shape dimorphism, iii) rates of evolution of shape dimorphism with magnitude of sexual size dimorphism iv) rates of evolution of shape dimorphism, and v) the percent of species in each clade with size dimorphism (see Table 2). This suggests that evolutionary changes in sexual size dimorphism among taxa are not related to shape dimorphism among taxa.

Table 2. Comparison between SSD and SShD rates of evolution, magnitude of sexual dimorphism and percent species with sexual dimorphism in each clade. Abbreviations: M_{SSD}/M_{SShD} = mean magnitude of SSD/SShD, $\%N_{SSD}/\%N_{SShD}$ = percent of species with SSD/SShD in each clade.

Factors	Beta	t	p
σ^2_{SSD} vs. σ^2_{SShD}	14.4593	1.167	0.295
σ^2_{SSD} vs. M_{SShD}	7.6294	0.619	0.562
σ^2_{SSD} vs. $\%N_{SShD}$	- 0.0036	- 0.505	0.634
σ^2_{SShD} vs. M_{SSD}	- 0.0010	- 0.288	0.784
σ^2_{SShD} vs. $\%N_{SSD}$	- 0.0001	- 1.969	0.105
M_{SSD} vs. M_{SShD}	0.0012	0.300	0.775
$\%N_{SSD}$ vs. $\%N_{SShD}$	- 0.0461	- 0.299	0.776

Additional clade-level descriptors.- Using PGLS we found significant correlations between i) rate of shape dimorphism and species diversity, ii) rate of shape dimorphism and species diversification rate, iii) age of clades and percent of species with shape dimorphism and iv) species diversity and diversification rate among hummingbird sub-clades (see Table 3). There was also a significant relationship between the rate of evolution of size dimorphism and percent of species with size dimorphism; though this relationship was driven by the *Mellisugini* clade. That the rate and magnitude of size dimorphism does not impact (nor is impacted by) these other higher-level descriptors is in contrast to that seen with shape dimorphism, and suggests that shape dimorphism has a relatively larger impact on species diversification and morphological diversity than does size dimorphism. Further, the significant associations between rates of evolution of shape dimorphism, rates of species diversification, species richness and clade age demonstrate that these factors are more influential in macroevolutionary patterns of diversity (Fig. 5).

Table 3. Results of PGLS hypothesis testing of sexual size (A) and shape (B) dimorphism with multiple factors. Significance between σ^2_{SSD} vs. %N_{SSD} is driven by the *Mellisugini* clade and thus does not represent a significant relationship between the factors. Abbreviations: %N_{SSD}/ %N_{SShD} = percent of species with SSD/SShD in each clade, D_{TIME} = rate of species diversification, N_{Clade} = species diversity, M_{SSD}/ M_{SShD} = mean magnitude of SSD/SShD

A. SSD	Beta	t	p
σ^2_{SSD} vs. Age _{SSD}	-0.0005	-0.0265	0.9798
σ^2_{SSD} vs. N _{Clade}	0.0123	0.1358	0.8972
σ^2_{SSD} vs. %N _{SSD}	-0.0042	-3.0528	0.0283*

Table 3 (continued) A. SSD	Beta	t	p
σ^2_{SSD} vs. D_{TIME}	18.2964	0.7487	0.4877
D_{TIME} vs. $\%N_{SSD}$	-6599.4	-1.7090	0.1481
Age_{Clade} vs. D_{TIME}	356.68	0.5715	0.5924
Age_{Clade} vs. N_{Clade}	1.8263	0.8597	0.4292
Age_{Clade} vs. $\%N_{SSD}$	-0.0376	-0.6633	0.5364
N_{Clade} vs. D_{TIME}	269.09	6.8614	0.0010*
σ^2_{SSD} vs. M_{SSD}	0.0589	0.3811	0.7188
Age_{Clade} vs. M_{SSD}	5.7129	1.9230	0.1125
M_{SSD} vs. N_{Clade}	-0.6117	-1.5942	0.1718
M_{SSD} vs. $\%N_{SSD}$	0.0072	0.6974	0.5166
M_{SSD} vs. D_{TIME}	-47.771	-0.6790	0.5273
N_{Clade} vs. $\%N_{SSD}$	-0.0121	-1.1778	0.2919

B. SShD	Beta	t	p
σ^2_{SShD} vs. Age_{Clade}	0.0006	0.984	0.3702
σ^2_{SShD} vs. N_{Clade}	0.0062	3.047	0.0285*
σ^2_{SShD} vs. $\%N_{SShD}$	0.0005	1.955	0.1079
σ^2_{SShD} vs. D_{TIME}	1.9500	4.494	0.0064 *
D_{TIME} vs. $\%N_{SSD}$	1757.2	1.637	0.1624
Age_{Clade} vs D_{TIME}	356.68	0.571	0.5924

Table 3 B. SShD (continued)	Beta	t	p
Age _{Clade} vs N _{Clade}	1.8263	0.859	0.4292
Age_{Clade} vs. %N_{SShD}	0.4335	4.486	0.0065*
N_{Clade} vs. D_{TIME}	269.09	6.861	0.0010*
σ^2_{SShD} vs. M _{SShD}	0.8817	2.4759	0.0561
Age _{Clade} vs. M _{SShD}	432.25	1.4732	0.2007
M _{SShD} vs. N _{Clade}	0.0014	0.4563	0.667
M _{SSD} vs. %N _{SShD}	0.0003	1.4157	0.2160
M _{SShD} vs. D _{TIME}	0.9166	1.2910	0.2532
N _{Clade} vs. %N _{SSD}	6.8674	1.9310	0.1114

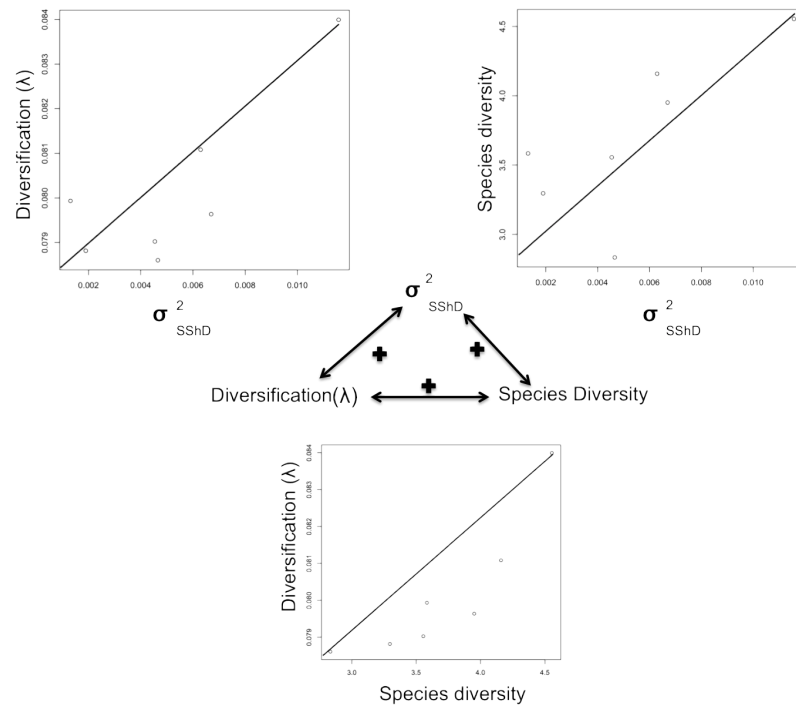


Figure 5. Positive correlations between rate of evolution of sexual shape dimorphism, species richness and rate of species diversification

Discussion

Rates of morphological evolution can differ between sets of related species (lineages), and is a major factor underlying and maintaining biodiversity. Many patterns stem from these differences, and it is expected that these patterns correlate with other clade-level patterns such as rates of species diversification, species richness, and clade age. Using bill morphology of 269 species of hummingbirds we quantified patterns of sexual size and shape dimorphism to shed light into causal mechanisms that may underlie patterns of the evolution of sexual dimorphism and ultimately, biodiversity. Interestingly, we found that rates of sexual size dimorphism do not differ between males and females and that the average magnitude of size dimorphism within clades was not greater in clades with higher rates of evolution of size dimorphism. Thus, a faster rate of sexual size dimorphism evolution does not necessarily equate to an increase in within-clade variation but rather, can simply mean that there are an increased number of phenotypic changes in those clades. One implication of this finding in hummingbirds is that size dimorphism is evolutionarily labile and exhibits a high variance, as a faster rate equates with an increased number of phenotypic changes but does not necessarily translate to increased magnitudes overall. Thus, at a macroevolutionary scale we might expect size dimorphism to be more common than shape dimorphism across species, because the rate of evolution of size dimorphism is significantly greater than the rate of evolution of shape dimorphism. Indeed, the majority of hummingbirds do exhibit size, but not shape dimorphism, lending support to this hypothesis.

Another interesting result from this study is that in all hummingbird lineages, the rate of evolution of sexual size dimorphism is higher than the rate of shape dimorphism. One reason this may be the case is if the strength of selection is stronger on size dimorphism than it is on shape dimorphism, due to the differential roles males and females have in reproduction and parental care, (Kaufman, 1996). This result may also reflect the evolutionary lability of sexual size dimorphism, or perhaps simply because size is a less complex characteristic than is shape. The faster rate of evolution of size dimorphism may also reflect physiological or developmental constraints on the evolution of shape changes that are not present in the evolution of size dimorphism.

We found no significant correlation between the mean magnitude of size dimorphism and the magnitude of shape dimorphism. Furthermore, the rates of evolution of sexual size dimorphism did not predict those of shape dimorphism (and vice versa). Together the absence of correlations suggests that changes in size and shape are unrelated to one another evolutionarily, which implies the decoupling at a macroevolutionary scale of the evolution of these traits. This is surprising, particularly since allometry (a relationship of changes in shape to overall size: Levinton, 1988; Mosimann, 1970) is found in many organisms, and is thus expected to generate macroevolutionary links between trends in size and shape evolution. However, many evolutionary forces are known to influence size and shape separately, and under such circumstances, rates of evolution of size and shape, (or size and shape dimorphism), may be expected to change independently of one another. For instance, the combination of genetic correlations between male and female size with directional sexual selection for larger male size can

cause the evolution of larger males relative to female body size (Abouheif and Fairbairn, 1997; Fairbairn, 1997; Fairbairn and Preziosi, 1994). Alternatively, when females are larger, selection for increased female fecundity can drive the pattern: larger females will have bigger or more eggs and a greater capacity to reproduce successfully (Darwin, 1871; Hughes and Hughes, 1986; Williams, 1966). Male-male combat may also impact the evolution of size dimorphism, because larger males are able to better compete for females than small males (Benson and Basolo, 2006).

Forces acting on shape alone may also drive patterns of shape dimorphism while not affecting size. For instance, a recent study looked at environmental gradients underlying sexual dimorphism and parallel evolution of a species of guppy *Poecilia reticulata* (Hendry et al., 2006), where populations experiencing high predation were made up of males with smaller heads and deeper caudal peduncles than females. It is also possible that sexual shape dimorphism is a pleiotropic effect where sex-linked genes determine the allocation of this trait differently in males and females. Any variation of these genes due to plasticity may then determine the genetic relationship of sexual shape dimorphism and differing conditions (Bonduriansky, 2006). In hummingbirds, it has been proposed that differences in the size and shape of the bill between the sexes reflect interspecific competition and adaptations to different food resources (Stiles, 1981, 1995b; Temeles, 1996; Temeles and Kress, 2003; Temeles et al., 2010). In this case, bill size and shape dimorphism likely reflects differences in the morphology of the flower from which each sex feeds, rather than one trait driving the evolution of the other. This would suggest that ecological factors greatly impact hummingbird diversification.

Perhaps most intriguing, we found a positive correlation between rates of species diversification, rates of sexual shape dimorphism, and species richness (Fig. 5). A number of causal mechanisms may drive these relationships. First, these positive correlations are consistent with the theory of ‘punctuated equilibrium,’ where higher rates of phenotypic evolution (in this case shape dimorphism) correlate with higher diversification rates. In hummingbirds, we could then expect that trends in mean morphology among the species of each clade will result from sequences of differential speciation or extinction (Damuth, 2001). Alternatively, such patterns may be the result of ecological mechanisms, such as the opening of new niches, or with radical changes in environment (Simpson, 1944, 1953). Indeed, it seems likely that ecological selection pressures influence the macroevolutionary patterns of hummingbird diversity, as bill morphology is a major cause of trophic differences either between species (Brown and Bowers, 1985; Montgomerie, 1984) or between the sexes (Darwin, 1874; Temeles et al., 2005; Temeles et al., 2000). In fact, several studies have documented a correlation between sexual dimorphism in bill curvature and foraging behaviors (Paton and Collins, 1989; Rico-G, 2008; Temeles and Kress, 2003; Temeles et al., 2002; Temeles et al., 2000). These observations lend support to the hypothesis that floral and bill morphology are tightly linked, such that a change in the flower morphology may lead to a concomitant change in bill morphology. When there is sexual dimorphism in the bills of hummingbirds, this can suggest that the sexes feed from different flowers, and that interspecific competition and niche partitioning are important in understanding macroevolutionary mechanisms underlying hummingbird diversity (Temeles and Kress,

2003; Temeles et al., 2000).

Understanding evolutionary change and the processes that drive these patterns is essential for understanding how biological diversity is generated and maintained. Here, we found that size and shape dimorphism are evolutionarily decoupled and that size dimorphism is evolutionarily more labile than is shape dimorphism. Further, we found that rates of evolution of shape dimorphism can predict rates of species diversification and that these both predict patterns of species richness. These findings elucidate macroevolutionary patterns of hummingbird diversity, highlight major processes that may underlie hummingbird diversity and suggest that, not only do further studies need to incorporate clade level descriptors such as species richness, rate of diversification and rate of morphological evolution, but that rates of sexual dimorphism evolution also play an important role in macroevolutionary patterns. Further studies need to account for the evolution of sexual dimorphism in order to create a fuller picture of the evolution of sexual dimorphism, and ultimately diversity.

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Appendix A

The average magnitude of sexual size dimorphism (SSD) and significance for each species. Bold denotes a significant presence of SSD.

Species	Mean Sq.	Approx. F	p	Magnitude of SSD
<i>Abeillia abeillei</i>	8.757	15.519	< 0.001	1.193
<i>Adelomyia melanogenys</i>	0.496	0.3379	0.5672	0.294
<i>Aglaeactis aliciae</i>	9.918	3.8706	0.0807	1.907
<i>Aglaeactis castelnaudii</i>	2.814	1.2105	0.2821	0.676
<i>Aglaeactis cupripennis</i>	22.110	0.8843	0.3582	2.013
<i>Aglaeactis pamela</i>	0.367	0.4773	0.4980	0.272
<i>Agelaiocercus coelestis</i>	16.790	15.6382	< 0.001	1.697
<i>Agelaiocercus kingi</i>	28.553	2.309	0.143	2.189
<i>Amazilia amabilis</i>	16.928	17.786	< 0.001	1.762
<i>Amazilia amazilia</i>	11.491	10.527	0.004	1.516
<i>Amazilia beryllina</i>	21.511	13.992	0.001	1.697
<i>Amazilia boucardi</i>	2.514	0.704	0.412	0.693
<i>Amazilia candida</i>	15.730	9.421	0.005	1.560
<i>Amazilia chionogaster</i>	3.039	1.258	0.275	0.733
<i>Amazilia cyanifrons</i>	4.900	6.300	0.024	1.123
<i>Amazilia cyanocephala</i>	4.174	2.751	0.105	0.617
<i>Amazilia cyanura</i>	4.510	3.994	0.061	1.036
<i>Amazilia decora</i>	21.880	14.758	0.001	2.003
<i>Amazilia edward</i>	0.664	1.033	0.321	0.334
<i>Amazilia fimbriata</i>	29.588	27.516	< 0.001	2.140
<i>Amazilia franciae</i>	7.985	2.244	0.147	1.107
<i>Amazilia lactea</i>	26.658	9.496	0.007	2.399
<i>Amazilia leucogaster</i>	10.519	5.240	0.037	1.646
<i>Amazilia rosenbergi</i>	0.003	0.001	0.970	0.021
<i>Amazilia rutila</i>	29.156	13.446	0.002	2.312
<i>Amazilia saucerrottei</i>	0.487	0.522	0.480	0.321
<i>Amazilia tobaci</i>	22.040	8.261	0.009	2.002
<i>Amazilia tzacatl</i>	26.722	9.376	0.005	1.930
<i>Amazilia versicolor</i>	16.394	16.942	< 0.001	1.520
<i>Amazilia violiceps</i>	18.159	8.581	0.009	1.945
<i>Amazilia viridicauda</i>	10.108	11.785	0.003	1.673
<i>Amazilia viridifrons</i>	5.100	1.994	0.170	0.875
<i>Amazilia viridigaster</i>	0.057	0.044	0.836	0.105
<i>Amazilia yucatanensis</i>	1.027	0.335	0.569	0.447

Appendix A (continued) Species	Mean Squares	Approx. F	P	Magnitude of SSD
<i>Androdon aequatorialis</i>	53.306	23.807	< 0.001	2.981
<i>Anthracothorax dominicus</i>	23.404	5.455	0.027	1.824
<i>Anthracothorax mango</i>	4.499	3.145	0.088	0.817
<i>Anthracothorax nigricollis</i>	10.460	6.753	0.016	1.284
<i>Anthracothorax prevostii</i>	3.004	1.008	0.323	0.604
<i>Anthracothorax veraguensis</i>	5.988	4.935	0.043	1.320
<i>Anthracothorax viridigula</i>	49.696	28.366	< 0.001	2.842
<i>Anthracothorax viridis</i>	40.372	32.874	< 0.001	2.408
<i>Archilochus alexandri</i>	33.165	19.070	< 0.001	0.693
<i>Archilochus colubris</i>	62.972	125.479	< 0.001	1.816
<i>Atthis Heliosa</i>	7.231	20.834	< 0.001	0.958
<i>Augastes scutatus</i>	0.347	0.654	0.432	0.318
<i>Avocettula recurvirostris</i>	0.064	0.065	0.805	0.163
<i>Boissonneaua flavescens</i>	0.235	0.319	0.578	0.203
<i>Boissonneaua jardini</i>	1.505	0.885	0.361	0.613
<i>Boissonneaua matthewsii</i>	1.792	1.189	0.290	0.602
<i>Calliphlox amethystina</i>	1.144	1.283	0.269	0.431
<i>Calliphlox bryantae</i>	3.936	6.626	0.017	0.794
<i>Calliphlox evelynae</i>	0.091	0.068	0.797	0.119
<i>Calliphlox mitchelli</i>	3.118	4.322	0.053	0.994
<i>Calothorax lucifer</i>	2.958	2.585	0.119	0.629
<i>Calothorax pulcher</i>	12.738	4.892	0.047	2.112
<i>Calypte anna</i>	14.860	14.741	0.001	1.403
<i>Calypte costae</i>	4.664	5.128	0.032	0.818
<i>Campylopterus cirrochloris</i>	7.049	4.813	0.039	1.243
<i>Campylopterus curvipennis</i>	11.731	4.280	0.052	1.467
<i>Campylopterus duidae</i>	11.169	10.124	0.006	1.585
<i>Campylopterus ensipennis</i>	18.584	2.454	0.133	1.846
<i>Campylopterus falcatus</i>	15.346	4.110	0.060	1.894
<i>Campylopterus hemileucurus</i>	15.774	3.948	0.062	1.785
<i>Campylopterus hyperythrus</i>	0.071	0.026	0.874	0.130
<i>Campylopterus largipennis</i>	21.011	11.606	0.003	1.955
<i>Campylopterus rufus</i>	6.180	2.322	0.139	0.910
<i>Campylopterus villavicencio</i>	19.356	6.717	0.016	1.773
<i>Chaetocercus astreans</i>	7.459	19.981	< 0.001	1.115
<i>Chaetocercus bombus</i>	2.580	6.552	0.023	0.810
<i>Chaetocercus heliodor</i>	8.158	7.389	0.011	1.027
<i>Chaetocercus jourdanii</i>	5.939	6.592	0.016	0.923
<i>Chaetocercus mulsanti</i>	15.844	13.059	0.001	1.365
<i>Chalcostigma herrani</i>	12.669	8.888	0.006	1.329

Appendix A (continued) Species	Mean Squares	Approx. F	P	Magnitude of SSD
<i>Chalcostigma heteropogon</i>	0.187	0.198	0.662	0.199
<i>Chalcostigma olivaceum</i>	0.104	0.040	0.846	0.184
<i>Chalcostigma ruficeps</i>	0.035	0.031	0.863	0.072
<i>Chalcostigma stanleyi</i>	4.338	6.169	0.020	0.819
<i>Chalybura buffonii</i>	0.930	0.559	0.460	0.348
<i>Chalybura urochrysia</i>	0.198	0.143	0.710	0.200
<i>Chlorostilbon assimilis</i>	7.865	7.651	0.012	1.225
<i>Chlorostilbon aureoventris</i>	38.189	16.614	< 0.001	2.490
<i>Chlorostilbon auriceps</i>	0.158	0.322	0.579	0.214
<i>Chlorostilbon canivetii</i>	4.625	4.591	0.043	0.878
<i>Chlorostilbon gibsoni</i>	7.925	13.685	0.001	1.040
<i>Chlorostilbon maugaeus</i>	1.614	1.706	0.200	0.422
<i>Chlorostilbon melanorhynchus</i>	0.039	0.052	0.823	0.088
<i>Chlorostilbon mellisugus</i>	1.792	2.333	0.139	0.524
<i>Chlorostilbon notatus</i>	10.543	12.564	0.002	1.385
<i>Chlorostilbon poortmani</i>	4.376	1.424	0.249	0.972
<i>Chlorostilbon ricordii</i>	9.785	12.087	0.002	1.319
<i>Chlorostilbon russatus</i>	7.360	8.973	0.009	1.279
<i>Chlorostilbon stenura</i>	8.839	4.961	0.038	1.289
<i>Chlorostilbon swainsonii</i>	6.554	6.574	0.019	1.110
<i>Chrysolampis mosquitos</i>	0.796	0.199	0.658	0.303
<i>Chrysuronina oenone</i>	14.384	18.941	< 0.001	1.583
<i>Clytolaema rubricauda</i>	0.885	0.838	0.371	0.411
<i>Coeligena bonapartei</i>	20.859	14.868	0.001	1.906
<i>Coeligena coeligena</i>	0.821	0.271	0.607	0.355
<i>Coeligena helianthea</i>	45.700	24.547	< 0.001	2.954
<i>Coeligena iris</i>	28.169	7.562	0.013	2.422
<i>Coeligena lutetiae</i>	43.857	13.680	0.002	2.894
<i>Coeligena phalerata</i>	5.011	0.582	0.454	0.955
<i>Coeligena torquata</i>	10.716	2.793	0.112	1.464
<i>Coeligena wilsoni</i>	1.471	0.637	0.438	0.626
<i>Colibri coruscans</i>	6.033	2.900	0.097	0.810
<i>Colibri delphinae</i>	3.063	1.069	0.310	0.619
<i>Colibri iolatus</i>	5.544	3.925	0.066	1.144
<i>Colibri serrirostris</i>	55.489	32.728	< 0.001	2.773
<i>Colibri thalassinus</i>	0.786	0.521	0.475	0.277
<i>Cyanthus latirostris</i>	12.936	9.294	0.006	1.534
<i>Cynanthus sordidus</i>	5.174	3.078	0.094	0.949
<i>Damophila julie</i>	11.933	9.944	0.005	1.430
<i>Discosura conversii</i>	2.326	4.864	0.038	0.623

Appendix A (continued) Species	Mean Squares	Approx. F	P	Magnitude of SSD
<i>Doricha eliza</i>	2.581	5.664	0.033	0.847
<i>Doricha enicura</i>	9.781	2.660	0.118	1.581
<i>Doryfera johannae</i>	40.606	34.115	< 0.001	2.551
<i>Doryfera ludoviciae</i>	115.571	14.017	0.001	3.964
<i>Elvira chionura</i>	12.237	3.706	0.069	1.528
<i>Elvira cupreiceps</i>	4.111	65.405	< 0.001	0.865
<i>Ensifera ensifera</i>	174.386	0.922	0.346	5.172
<i>Eriocnemis alinae</i>	8.869	8.879	0.007	1.243
<i>Eriocnemis cupreovertris</i>	14.471	21.773	< 0.001	1.768
<i>Eriocnemis derbyi</i>	4.298	1.976	0.176	0.906
<i>Eriocnemis glaucopoides</i>	4.878	2.334	0.158	1.353
<i>Eriocnemis luciani</i>	3.155	3.437	0.074	0.646
<i>Eriocnemis mosquera</i>	9.308	6.750	0.017	1.301
<i>Eriocnemis nigriventris</i>	1.476	2.592	0.125	0.546
<i>Eriocnemis vestitus</i>	3.011	1.984	0.170	0.647
<i>Eugenes fulgens</i>	5.945	0.721	0.401	0.827
<i>Eulampis holosericeus</i>	24.126	5.993	0.021	1.788
<i>Eulampis jugularis</i>	78.982	23.862	< 0.001	3.423
<i>Eupetomena macroura</i>	14.427	7.457	0.010	1.367
<i>Eupherusa cyanophrys</i>	0.075	0.120	0.733	0.135
<i>Eupherusa eximia</i>	12.525	6.537	0.019	1.509
<i>Eupherusa nigriventris</i>	0.003	0.010	0.921	0.029
<i>Eupherusa poliocerca</i>	0.061	0.034	0.857	0.145
<i>Eutoxeres aquila</i>	1.580	0.527	0.476	0.525
<i>Eutoxeres condamini</i>	1.400	0.708	0.409	0.477
<i>Florisuga mellivora</i>	24.278	12.631	0.002	1.985
<i>Glaucis aenea</i>	7.030	4.727	0.044	1.218
<i>Glaucis hirsuta affinis</i>	28.618	25.894	< 0.001	2.233
<i>Haplophadia aureliae</i>	17.665	25.516	< 0.001	1.694
<i>Heliactin bilophum/cornutus</i>	3.269	0.300	0.591	0.848
<i>Heliangelus amethysticollis</i>	2.328	2.793	0.111	0.673
<i>Heliangelus exortis</i>	2.769	2.693	0.117	0.748
<i>Heliangelus mavors</i>	0.186	0.190	0.668	0.213
<i>Heliangelus regalis</i>	6.452	13.387	0.003	1.270
<i>Heliangelus spencei</i>	1.589	1.483	0.238	0.551
<i>Heliangelus strophianus</i>	0.008	0.012	0.914	0.045
<i>Heliangelus viola</i>	8.672	8.218	0.010	1.317
<i>Heliodoxa aurescens</i>	15.558	12.388	0.002	1.659
<i>Heliodoxa branickii</i>	10.682	9.339	0.006	1.394
<i>Heliodoxa gularis</i>	16.647	9.534	0.006	1.799

Appendix A (continued) Species	Mean Squares	Approx. F	P	Magnitude of SSD
<i>Heliodoxa imperatrix</i>	1.797	1.464	0.252	0.746
<i>Heliodoxa jacula</i>	2.815	0.803	0.381	0.715
<i>Heliodoxa leadbeateri</i>	24.329	8.710	0.006	1.860
<i>Heliodoxa runinoides</i>	19.118	6.253	0.021	1.872
<i>Heliodoxa schreibersii</i>	27.490	10.818	0.003	2.063
<i>Heliodoxa xanthogonys</i>	60.119	77.098	< 0.001	3.563
<i>Heliomaster constantii</i>	8.729	2.189	0.148	1.025
<i>Heliomaster furcifer</i>	11.189	4.903	0.041	1.537
<i>Heliomaster squamosus</i>	3.293	1.637	0.230	1.111
<i>Heliothryx aurita</i>	12.717	27.450	< 0.001	1.456
<i>Heliothryx barroti</i>	1.508	1.891	0.181	0.475
<i>Hylocharis chrysura</i>	9.378	5.768	0.022	1.079
<i>Hylocharis cyanus</i>	3.563	2.037	0.165	0.695
<i>Hylocharis eliciae</i>	0.786	1.148	0.296	0.370
<i>Hylocharis grayi</i>	3.359	3.752	0.063	0.661
<i>Hylocharis leucotis</i>	4.513	3.099	0.095	0.955
<i>Hylocharis sapphirina</i>	2.144	2.261	0.147	0.600
<i>Hylocharis xantusi</i>	0.027	0.023	0.881	0.079
<i>Klais guimeti</i>	2.159	3.594	0.073	0.642
<i>Lafresnaya lafresnayi</i>	0.135	0.011	0.916	0.139
<i>Lampornis amethystinus</i>	4.992	2.064	0.179	1.243
<i>Lampornis calolaema</i>	18.960	17.830	< 0.001	1.743
<i>Lampornis castaneoventris</i>	8.783	13.367	0.002	1.362
<i>Lampornis clemenciae</i>	64.198	34.146	< 0.001	3.036
<i>Lampornis rhami</i>	2.785	2.684	0.115	0.681
<i>Lampornis viridipallens</i>	11.048	12.610	0.001	1.115
<i>Lepidopyga coeruleogularis</i>	8.645	8.025	0.011	1.322
<i>Lepidopyga goudoti</i>	0.037	0.024	0.878	0.089
<i>Lesbia nuna</i>	1.440	0.890	0.353	0.428
<i>Lesbia victoriae</i>	0.236	0.159	0.695	0.218
<i>Leucippus fallax</i>	8.411	5.927	0.026	1.297
<i>Leucippus taczanowskii</i>	2.574	0.552	0.465	0.676
<i>Loddigesia mirabilis</i>	29.436	11.100	0.008	3.177
<i>Lophornis adorabilis</i>	0.044	0.051	0.824	0.088
<i>Lophornis chalybea</i>	0.094	0.046	0.833	0.149
<i>Lophornis delattrei</i>	6.663	6.440	0.017	0.978
<i>Lophornis helenae</i>	0.649	1.723	0.204	0.336
<i>Lophornis ornatus</i>	0.388	0.855	0.364	0.240
<i>Mellisuga helenae</i>	7.454	11.726	0.002	0.967
<i>Mellisuga minima</i>	4.562	11.677	0.002	0.855

Appendix A (continued) Species	Mean Squares	Approx. F	P	Magnitude of SSD
<i>Metallura aeneocauda</i>	5.671	6.585	0.018	1.015
<i>Metallura eupogon</i>	0.216	0.175	0.680	0.199
<i>Metallura odomae</i>	3.034	2.045	0.181	0.969
<i>Metallura phoebe</i>	1.150	0.830	0.373	0.448
<i>Metallura tyrianthina</i>	8.584	2.380	0.134	1.079
<i>Metallura williami</i>	11.722	4.884	0.037	1.347
<i>Microstilbon burmeisteri</i>	6.271	15.129	< 0.001	0.605
<i>Myrmia micrura</i>	3.836	5.127	0.038	0.947
<i>Myrtis fanny</i>	0.293	0.197	0.660	0.225
<i>Ocreatus underwoodii</i>	12.435	12.463	0.002	1.577
<i>Opisthoprora euryptera</i>	2.244	0.196	0.664	0.696
<i>Oreonympha nobilis</i>	0.079	0.039	0.846	0.128
<i>Oreotrochilus estella</i>	0.951	0.421	0.524	0.423
<i>Oreotrochilus leucopleurus</i>	12.030	7.455	0.010	1.213
<i>Oreotrochilus melanogaster</i>	1.051	0.168	0.689	0.572
<i>Orthrorhynchus cristatus</i>	7.258	2.668	0.117	1.133
<i>Oxypogon guerinii</i>	0.012	0.031	0.864	0.058
<i>Panterpe insignis</i>	13.434	31.641	< 0.001	1.647
<i>Patagona gigas</i>	5.086	0.471	0.498	0.821
<i>Phaeochroa cuvieri</i>	3.271	1.263	0.276	0.813
<i>Phaethornis anthophilus</i>	34.651	11.820	0.003	2.572
<i>Phaethornis augusti</i>	27.812	13.108	0.001	2.153
<i>Phaethornis bourcierii</i>	73.351	16.122	< 0.001	2.895
<i>Phaethornis eurynome</i>	88.863	15.497	0.001	3.965
<i>Phaethornis griseogularis</i>	0.384	0.378	0.546	0.291
<i>Phaethornis guy</i>	162.242	8.298	0.009	5.454
<i>Phaethornis hispidus</i>	6.693	1.752	0.198	1.013
<i>Phaethornis koepckae</i>	62.900	26.584	< 0.001	3.111
<i>Phaethornis longuemareus</i>	17.440	15.987	0.001	1.940
<i>Phaethornis malaris</i>	58.310	30.222	< 0.001	3.509
<i>Phaethornis philippii</i>	77.046	72.266	< 0.001	3.326
<i>Phaethornis pretrei</i>	39.769	33.705	< 0.001	2.834
<i>Phaethornis ruber</i>	26.115	9.773	0.004	1.977
<i>Phaethornis rupurmi</i>	0.495	0.515	0.481	0.300
<i>Phaethornis squalidus</i>	3.423	1.264	0.275	0.856
<i>Phaethornis striigularis</i>	17.733	7.707	0.011	1.719
<i>Phaethornis subochraceus</i>	12.882	0.944	0.347	1.910
<i>Phaethornis superciliosus</i>	3.453	3.016	0.100	0.831
<i>Phaethornis syrmatophorus</i>	103.405	34.325	< 0.001	4.097
<i>Phaethornis yaruqui</i>	122.847	24.211	< 0.001	4.982

Appendix A (continued) Species	Mean Squares	Approx. F	P	Magnitude of SSD
<i>Phlogophilus harterti</i>	1.015	1.593	0.220	0.452
<i>Polytmus guainumbi</i>	25.627	11.100	0.003	2.067
<i>Polytmus milleri</i>	0.040	0.082	0.777	0.089
<i>Polytmus theresiae</i>	1.986	2.435	0.128	0.493
<i>Popelairia langsdorffi</i>	0.210	0.224	0.641	0.200
<i>Pterophanes cyanopterus</i>	2.146	0.657	0.426	0.590
<i>Ramphodon naevius</i>	60.302	7.718	0.009	2.727
<i>Ramphomicron microrhynchum</i>	0.430	0.767	0.390	0.263
<i>Rhodopis vesper</i>	34.673	3.506	0.071	2.246
<i>Sappho sparganura</i>	0.524	0.212	0.649	0.283
<i>Schistes geoffroyi</i>	0.049	0.020	0.889	0.084
<i>Selasphorus flammula</i>	4.499	7.610	0.009	0.689
<i>Selasphorus platycercus</i>	11.400	8.092	0.009	1.308
<i>Selasphorus rufus</i>	55.288	53.935	< 0.001	2.818
<i>Selasphorus sasin</i>	0.065	0.054	0.817	0.073
<i>Selasphorus scintilla</i>	2.443	6.788	0.016	0.626
<i>Sephanoides sephanoides</i>	17.097	15.595	< 0.001	1.421
<i>Stellula calliope</i>	4.896	5.838	0.021	0.748
<i>Stephanoxis lalandi</i>	9.291	3.239	0.082	1.095
<i>Sternoclyta cyanopectus</i>	56.112	3.928	0.063	3.350
<i>Thalurania colombica</i>	3.811	5.945	0.025	0.873
<i>Thalurania fannyi</i>	5.030	4.138	0.057	1.003
<i>Thalurania furcata</i>	10.325	4.134	0.054	1.295
<i>Thalurania glaucopis</i>	0.032	0.018	0.895	0.078
<i>Thaumastura cora</i>	6.386	14.029	< 0.001	0.865
<i>Therentes leucurus</i>	2.179	0.923	0.348	0.632
<i>Threnetes ruckeri</i>	21.115	16.546	0.001	2.055
<i>Tilmatura dupontii</i>	3.503	8.499	0.007	0.721
<i>Topaza pella</i>	3.130	1.787	0.194	0.708
<i>Trochilus polytmus</i>	3.480	3.480	0.073	1.095
<i>Urochroa bougueri</i>	8.976	3.653	0.072	1.367
<i>Urosticte benjamini</i>	19.739	3.304	0.087	2.041
<i>Urosticte ruficrissa</i>	0.305	0.154	0.700	0.262

Appendix B

The average magnitude of sexual shape dimorphism (SShD) and significance for each species. Bold denotes a significant presence of SShD.

Species	Mean Sq.	Approx. F	p	Magnitude of SShD
<i>Abeillia abeillei</i>	0.011	1.670	0.130	0.046
<i>Adelomyia melanogenys</i>	0.014	1.746	0.148	0.050
<i>Aglaeactis aliciae</i>	0.003	0.895	0.424	0.036
<i>Aglaeactis castelnaudii</i>	0.003	0.839	0.492	0.023
<i>Aglaeactis cupripennis</i>	0.007	1.012	0.336	0.037
<i>Aglaeactis pamela</i>	0.002	0.667	0.660	0.021
<i>Agelaiocercus coelestis</i>	0.012	1.455	0.210	0.047
<i>Agelaiocercus kingi</i>	0.012	1.558	0.185	0.047
<i>Amazilia amabilis</i>	0.006	2.601	0.039	0.035
<i>Amazilia amazilia</i>	0.005	1.546	0.161	0.033
<i>Amazilia beryllina</i>	0.004	1.336	0.230	0.024
<i>Amazilia boucardi</i>	0.003	1.011	0.377	0.026
<i>Amazilia candida</i>	0.003	0.716	0.534	0.024
<i>Amazilia chionogaster</i>	0.002	0.702	0.631	0.019
<i>Amazilia cyanifrons</i>	0.001	0.373	0.902	0.020
<i>Amazilia cyanocephala</i>	0.004	0.607	0.585	0.019
<i>Amazilia cyanura</i>	0.012	3.594	0.018	0.055
<i>Amazilia decora</i>	0.003	1.382	0.227	0.024
<i>Amazilia edward</i>	0.003	1.256	0.266	0.024
<i>Amazilia fimbriata</i>	0.004	0.964	0.415	0.025
<i>Amazilia franciae</i>	0.001	0.360	0.924	0.013
<i>Amazilia lactea</i>	0.005	1.299	0.244	0.032
<i>Amazilia leucogaster</i>	0.004	2.064	0.073	0.034
<i>Amazilia rosenbergi</i>	0.004	1.337	0.236	0.027
<i>Amazilia rutila</i>	0.023	3.598	0.011	0.065
<i>Amazilia saucerrottei</i>	0.003	0.851	0.491	0.025
<i>Amazilia tobaci</i>	0.009	3.341	0.011	0.040
<i>Amazilia tzacatl</i>	0.001	0.441	0.861	0.014
<i>Amazilia versicolor</i>	0.034	6.301	0.002	0.071
<i>Amazilia violiceps</i>	0.004	0.582	0.541	0.028
<i>Amazilia viridicauda</i>	0.002	0.676	0.614	0.024
<i>Amazilia viridifrons</i>	0.002	0.711	0.570	0.017
<i>Amazilia viridigaster</i>	0.008	2.324	0.054	0.039
<i>Amazilia yucatanensis</i>	0.003	1.536	0.173	0.026
<i>Androdon aequatorialis</i>	0.004	1.376	0.244	0.026

Appendix B (continued) Species	Mean Sq.	Approx. F	p	Magnitude of SS_{hD}
<i>Anthracothorax dominicus</i>	0.009	1.616	0.186	0.037
<i>Anthracothorax mango</i>	0.004	1.429	0.209	0.024
<i>Anthracothorax nigricollis</i>	0.006	1.796	0.121	0.032
<i>Anthracothorax prevostii</i>	0.003	1.584	0.168	0.021
<i>Anthracothorax veraguensis</i>	0.005	1.669	0.179	0.038
<i>Anthracothorax viridigula</i>	0.003	1.309	0.258	0.022
<i>Anthracothorax viridis</i>	0.002	0.862	0.503	0.015
<i>Archilochus alexandri</i>	0.006	1.709	0.910	0.010
<i>Archilochus colubris</i>	0.014	2.944	0.047	0.027
<i>Atthis Heliosa</i>	0.003	0.452	0.850	0.018
<i>Augastes scutatus</i>	0.002	0.629	0.661	0.025
<i>Avocettula recurvirostris</i>	0.018	2.143	0.118	0.086
<i>Boissonneaua flavescens</i>	0.002	0.532	0.774	0.021
<i>Boissonneaua jardini</i>	0.002	1.270	0.258	0.024
<i>Boissonneaua matthewsii</i>	0.015	2.054	0.107	0.056
<i>Calliphlox amethystina</i>	0.002	0.296	0.840	0.017
<i>Calliphlox bryantae</i>	0.002	0.454	0.809	0.016
<i>Calliphlox evelynae</i>	0.003	0.750	0.594	0.021
<i>Calliphlox mitchelli</i>	0.003	0.871	0.458	0.032
<i>Calothorax lucifer</i>	0.002	0.410	0.768	0.017
<i>Calothorax pulcher</i>	0.003	1.935	0.085	0.036
<i>Calypte anna</i>	0.009	1.624	0.168	0.035
<i>Calypte costae</i>	0.001	0.562	0.750	0.015
<i>Campylopterus cirrochloris</i>	0.001	0.338	0.910	0.017
<i>Campylopterus curvipennis</i>	0.006	0.634	0.635	0.034
<i>Campylopterus duidae</i>	0.005	2.755	0.014	0.035
<i>Campylopterus ensipennis</i>	0.005	1.807	0.096	0.034
<i>Campylopterus falcatus</i>	0.002	0.724	0.552	0.021
<i>Campylopterus hemileucurus</i>	0.008	2.442	0.008	0.040
<i>Campylopterus hyperythrus</i>	0.003	1.410	0.191	0.026
<i>Campylopterus largipennis</i>	0.005	1.678	0.145	0.032
<i>Campylopterus rufus</i>	0.004	1.120	0.313	0.025
<i>Campylopterus villavicencio</i>	0.002	0.984	0.353	0.021
<i>Chaetocercus astreans</i>	0.003	1.261	0.256	0.022
<i>Chaetocercus bombus</i>	0.009	2.370	0.064	0.048
<i>Chaetocercus heliodor</i>	0.002	0.944	0.446	0.018
<i>Chaetocercus jourdanii</i>	0.008	1.480	0.212	0.036
<i>Chaetocercus mulsanti</i>	0.004	1.249	0.280	0.022
<i>Chalcostigma herrani</i>	0.015	2.354	0.042	0.047
<i>Chalcostigma heteropogon</i>	0.008	0.880	0.450	0.041

Appendix B (continued) Species	Mean Sq.	Approx. F	p	Magnitude of SShD
<i>Chalcostigma olivaceum</i>	0.037	1.332	0.255	0.110
<i>Chalcostigma ruficeps</i>	0.006	0.393	0.862	0.032
<i>Chalcostigma stanleyi</i>	0.006	0.530	0.773	0.033
<i>Chalybura buffonii</i>	0.003	0.718	0.582	0.020
<i>Chalybura urochrysia</i>	0.003	1.319	0.245	0.028
<i>Chlorostilbon assimilis</i>	0.017	3.566	0.007	0.057
<i>Chlorostilbon aureoventris</i>	0.006	2.427	0.066	0.033
<i>Chlorostilbon auriceps</i>	0.005	1.909	0.100	0.041
<i>Chlorostilbon canivetii</i>	0.005	1.036	0.357	0.028
<i>Chlorostilbon gibsoni</i>	0.003	0.806	0.542	0.021
<i>Chlorostilbon maugaeus</i>	0.004	0.500	0.720	0.021
<i>Chlorostilbon melanorhynchus</i>	0.012	3.192	0.019	0.049
<i>Chlorostilbon mellisugus</i>	0.010	1.681	0.163	0.040
<i>Chlorostilbon notatus</i>	0.006	1.058	0.338	0.035
<i>Chlorostilbon poortmani</i>	0.002	0.478	0.738	0.020
<i>Chlorostilbon ricordii</i>	0.001	0.288	0.911	0.016
<i>Chlorostilbon russatus</i>	0.009	2.538	0.053	0.045
<i>Chlorostilbon stenura</i>	0.016	3.902	0.014	0.055
<i>Chlorostilbon swainsonii</i>	0.018	1.462	0.239	0.060
<i>Chrysolampis mosquitus</i>	0.056	8.088	<0.001	0.082
<i>Chrysuronia oenone</i>	0.002	0.305	0.822	0.020
<i>Clytolaema rubricauda</i>	0.003	0.570	0.713	0.025
<i>Coeligena bonapartei</i>	0.002	1.270	0.271	0.021
<i>Coeligena coeligena</i>	0.001	0.194	0.951	0.010
<i>Coeligena helianthea</i>	0.005	2.351	0.061	0.029
<i>Coeligena iris</i>	0.004	1.210	0.292	0.030
<i>Coeligena lutetiae</i>	0.001	0.589	0.680	0.014
<i>Coeligena phalerata</i>	0.021	3.044	0.035	0.063
<i>Coeligena torquata</i>	0.007	5.567	0.004	0.038
<i>Coeligena wilsoni</i>	0.002	1.739	0.103	0.020
<i>Colibri coruscans</i>	0.010	2.456	0.047	0.033
<i>Colibri delphinae</i>	0.023	4.386	0.001	0.057
<i>Colibri iolatus</i>	0.002	0.734	0.620	0.025
<i>Colibri serrirostris</i>	0.002	0.695	0.604	0.018
<i>Colibri thalassinus</i>	0.011	2.497	0.054	0.034
<i>Cyanthus latirostris</i>	0.004	1.699	0.138	0.027
<i>Cyanthus sordidus</i>	0.008	1.832	0.152	0.037
<i>Damophila julie</i>	0.004	0.946	0.430	0.027
<i>Discosura conversii</i>	0.006	0.534	0.693	0.032
<i>Doricha eliza</i>	0.003	1.328	0.234	0.026

Appendix B (continued) Species	Mean Sq.	Approx. F	p	Magnitude of SS_{hD}
<i>Doricha enicura</i>	0.001	0.940	0.458	0.020
<i>Doryfera johannae</i>	0.001	0.795	0.522	0.016
<i>Doryfera ludoviciae</i>	0.001	0.730	0.555	0.012
<i>Elvira chionura</i>	0.020	1.868	0.130	0.063
<i>Elvira cupreiceps</i>	0.003	0.396	0.856	0.027
<i>Ensifera ensifera</i>	0.001	0.175	0.983	0.007
<i>Eriocnemis alinae</i>	0.004	1.192	0.276	0.029
<i>Eriocnemis cupreovertris</i>	0.010	2.161	0.098	0.047
<i>Eriocnemis derbyi</i>	0.008	0.947	0.427	0.041
<i>Eriocnemis glaucopoides</i>	0.003	0.951	0.410	0.033
<i>Eriocnemis luciani</i>	0.002	0.831	0.519	0.017
<i>Eriocnemis mosquera</i>	0.007	1.061	0.353	0.038
<i>Eriocnemis nigriventris</i>	0.001	0.289	0.913	0.013
<i>Eriocnemis vestitus</i>	0.007	1.595	0.171	0.032
<i>Eugenes fulgens</i>	0.002	1.016	0.378	0.019
<i>Eulampis holosericeus</i>	0.011	3.275	0.015	0.038
<i>Eulampis jugularis</i>	0.015	3.099	0.014	0.051
<i>Eupetomena macroura</i>	0.006	1.670	0.133	0.029
<i>Eupherusa cyanophrys</i>	0.003	0.377	0.837	0.028
<i>Eupherusa eximia</i>	0.007	0.823	0.431	0.037
<i>Eupherusa nigriventris</i>	0.003	1.137	0.325	0.031
<i>Eupherusa poliocerca</i>	0.009	1.497	0.202	0.057
<i>Eutoxeres aquila</i>	0.004	0.883	0.470	0.028
<i>Eutoxeres condamini</i>	0.006	0.907	0.413	0.032
<i>Florisuga mellivora</i>	0.024	1.516	0.192	0.063
<i>Glaucis aenea</i>	0.006	1.789	0.147	0.036
<i>Glaucis hirsuta affinis</i>	0.015	3.808	0.026	0.054
<i>Haplophadia aureliae</i>	0.006	0.947	0.411	0.033
<i>Heliactin bilophum/cornutus</i>	0.008	1.098	0.346	0.044
<i>Helianthus amethysticollis</i>	0.014	2.183	0.103	0.053
<i>Helianthus exortis</i>	0.004	0.866	0.471	0.028
<i>Helianthus mavors</i>	0.004	1.464	0.189	0.032
<i>Helianthus regalis</i>	0.005	1.383	0.235	0.036
<i>Helianthus spencei</i>	0.003	0.675	0.561	0.026
<i>Helianthus strophianus</i>	0.016	2.508	0.083	0.064
<i>Helianthus viola</i>	0.004	1.054	0.363	0.030
<i>Heliodoxa aurescens</i>	0.018	1.824	0.149	0.058
<i>Heliodoxa branickii</i>	0.018	3.240	0.027	0.058
<i>Heliodoxa gularis</i>	0.005	1.753	0.142	0.033
<i>Heliodoxa imperatrix</i>	0.005	1.215	0.283	0.041

Appendix B (continued) Species	Mean Sq.	Approx. F	p	Magnitude of SShD
<i>Heliodoxa jacula</i>	0.007	1.340	0.230	0.036
<i>Heliodoxa leadbeateri</i>	0.032	2.616	0.051	0.069
<i>Heliodoxa runinoides</i>	0.003	0.567	0.697	0.024
<i>Heliodoxa schreibersii</i>	0.003	0.426	0.806	0.021
<i>Heliodoxa xanthogonys</i>	0.010	2.580	0.027	0.047
<i>Heliomaster constantii</i>	0.002	1.232	0.274	0.015
<i>Heliomaster furcifer</i>	0.001	0.569	0.793	0.016
<i>Heliomaster squamosus</i>	0.004	1.010	0.358	0.040
<i>Heliothryx aurita</i>	0.026	3.609	0.035	0.065
<i>Heliothryx barroti</i>	0.005	0.913	0.434	0.029
<i>Hylocharis chrysura</i>	0.002	0.722	0.595	0.017
<i>Hylocharis cyanus</i>	0.018	2.787	0.037	0.049
<i>Hylocharis eliciae</i>	0.004	0.819	0.503	0.027
<i>Hylocharis grayi</i>	0.017	3.380	0.021	0.049
<i>Hylocharis leucotis</i>	0.007	1.709	0.162	0.039
<i>Hylocharis sapphirina</i>	0.008	1.834	0.118	0.037
<i>Hylocharis xantusii</i>	0.002	0.447	0.845	0.022
<i>Klais guimeti</i>	0.003	0.628	0.639	0.024
<i>Lafresnaya lafresnayi</i>	0.011	1.877	0.116	0.040
<i>Lampornis amethystinus</i>	0.006	1.659	0.189	0.044
<i>Lampornis calolaema</i>	0.003	0.719	0.522	0.026
<i>Lampornis castaneoventris</i>	0.007	2.312	0.076	0.039
<i>Lampornis clemenciae</i>	0.034	9.025	<0.001	0.070
<i>Lampornis rhami</i>	0.005	1.058	0.347	0.028
<i>Lampornis viridipallens</i>	0.003	0.949	0.400	0.018
<i>Lepidopyga coeruleogularis</i>	0.008	3.093	0.007	0.042
<i>Lepidopyga goudoti</i>	0.008	3.183	0.022	0.042
<i>Lesbia nuna</i>	0.025	2.799	0.020	0.057
<i>Lesbia victoriae</i>	0.008	1.412	0.225	0.041
<i>Leucippus fallax</i>	0.005	1.367	0.245	0.033
<i>Leucippus taczanowskii</i>	0.014	2.279	0.093	0.050
<i>Loddigesia mirabilis</i>	0.005	1.087	0.359	0.042
<i>Lophornis adorabilis</i>	0.010	1.593	0.150	0.044
<i>Lophornis chalybea</i>	0.005	0.520	0.703	0.035
<i>Lophornis delattrei</i>	0.038	2.559	0.044	0.077
<i>Lophornis helenae</i>	0.004	0.387	0.810	0.026
<i>Lophornis ornatus</i>	0.002	0.239	0.971	0.018
<i>Mellisuga helenae</i>	0.002	0.878	0.470	0.018
<i>Mellisuga minima</i>	0.021	4.965	0.005	0.058
<i>Metallura aeneocauda</i>	0.003	1.100	0.341	0.025

Appendix B (continued) Species	Mean Sq.	Approx. F	p	Magnitude of SS_{hD}
<i>Metallura eupogon</i>	0.007	1.201	0.304	0.037
<i>Metallura odomae</i>	0.003	0.255	0.952	0.029
<i>Metallura phoebe</i>	0.004	0.482	0.790	0.028
<i>Metallura tyrianthina</i>	0.005	0.778	0.537	0.026
<i>Metallura williami</i>	0.015	2.249	0.064	0.049
<i>Microstilbon burmeisteri</i>	0.006	1.083	0.325	0.019
<i>Myrmia micrura</i>	0.002	0.747	0.573	0.023
<i>Myrtis fanny</i>	0.002	0.647	0.634	0.019
<i>Ocreatus underwoodii</i>	0.016	2.027	0.111	0.057
<i>Opisthoprora euryptera</i>	0.011	0.409	0.709	0.050
<i>Oreonympha nobilis</i>	0.003	0.618	0.610	0.023
<i>Oreotrochilus estella</i>	0.007	0.957	0.415	0.038
<i>Oreotrochilus leucopleurus</i>	0.016	1.785	0.143	0.044
<i>Oreotrochilus melanogaster</i>	0.006	1.236	0.283	0.044
<i>Orthrorhynchus cristatus</i>	0.037	5.396	0.005	0.082
<i>Oxypogon guerinii</i>	0.014	1.498	0.186	0.065
<i>Panterpe insignis</i>	0.005	1.390	0.211	0.035
<i>Patagona gigas</i>	0.004	1.184	0.307	0.025
<i>Phaeochroa cuvieri</i>	0.005	1.364	0.226	0.033
<i>Phaethornis anthophilus</i>	0.010	1.833	0.150	0.046
<i>Phaethornis augusti</i>	0.004	2.326	0.047	0.028
<i>Phaethornis bourcierii</i>	0.005	1.090	0.317	0.024
<i>Phaethornis eurynome</i>	0.017	1.195	0.279	0.054
<i>Phaethornis griseogularis</i>	0.009	2.925	0.020	0.046
<i>Phaethornis guy</i>	0.005	2.197	0.092	0.031
<i>Phaethornis hispidus</i>	0.026	3.630	0.030	0.063
<i>Phaethornis koepckae</i>	0.022	6.535	0.004	0.061
<i>Phaethornis longuemareus</i>	0.003	0.478	0.664	0.026
<i>Phaethornis malaris</i>	0.001	0.551	0.602	0.017
<i>Phaethornis philippii</i>	0.004	1.272	0.265	0.025
<i>Phaethornis pretrei</i>	0.005	2.102	0.104	0.033
<i>Phaethornis ruber</i>	0.001	0.454	0.765	0.015
<i>Phaethornis rupurmi</i>	0.003	1.137	0.301	0.025
<i>Phaethornis squalidus</i>	0.001	0.226	0.923	0.016
<i>Phaethornis striigularis</i>	0.004	0.874	0.458	0.026
<i>Phaethornis subochraceus</i>	0.002	0.563	0.621	0.026
<i>Phaethornis superciliosus</i>	0.002	1.281	0.259	0.022
<i>Phaethornis syrmatophorus</i>	0.003	1.809	0.084	0.023
<i>Phaethornis yaruqui</i>	0.002	2.688	0.033	0.022
<i>Phlogophilus harterti</i>	0.001	0.809	0.577	0.018

Appendix B (continued) Species	Mean Sq.	Approx. F	p	Magnitude of SShD
<i>Polytmus guainumbi</i>	0.010	2.541	0.051	0.041
<i>Polytmus milleri</i>	0.005	1.659	0.161	0.031
<i>Polytmus theresiae</i>	0.004	1.788	0.105	0.022
<i>Popelairia langsdorffi</i>	0.011	1.116	0.335	0.045
<i>Pterophanes cyanopterus</i>	0.021	4.638	0.005	0.060
<i>Ramphodon naevius</i>	0.032	4.957	0.009	0.067
<i>Ramphomicron microrhynchum</i>	0.036	2.122	0.069	0.080
<i>Rhodopis vesper</i>	0.003	1.860	0.115	0.023
<i>Sappho sparganura</i>	0.004	0.506	0.648	0.025
<i>Schistes geoffroyi</i>	0.013	1.712	0.153	0.043
<i>Selasphorus flammula</i>	0.006	1.235	0.279	0.025
<i>Selasphorus platycercus</i>	0.002	0.709	0.587	0.016
<i>Selasphorus rufus</i>	0.004	1.637	0.139	0.025
<i>Selasphorus sasin</i>	0.004	0.947	0.394	0.019
<i>Selasphorus scintilla</i>	0.015	3.005	0.036	0.044
<i>Sephanoides sephanoides</i>	0.019	4.238	0.004	0.048
<i>Stellula calliope</i>	0.007	1.302	0.249	0.030
<i>Stephanoxis lalandi</i>	0.013	1.810	0.127	0.041
<i>Sternoclyta cyanopectus</i>	0.014	2.271	0.092	0.053
<i>Thalurania colombica</i>	0.002	0.553	0.734	0.021
<i>Thalurania fannyi</i>	0.001	0.461	0.828	0.015
<i>Thalurania furcata</i>	0.009	1.619	0.176	0.039
<i>Thalurania glaucopis</i>	0.011	1.613	0.177	0.049
<i>Thaumastura cora</i>	0.011	1.380	0.223	0.037
<i>Therentes leucurus</i>	0.007	1.092	0.328	0.036
<i>Threnetes ruckeri</i>	0.042	6.685	0.002	0.093
<i>Tilmatura dupontii</i>	0.005	1.448	0.196	0.027
<i>Topaza pella</i>	0.007	1.400	0.222	0.035
<i>Trochilus polytmus</i>	0.011	2.706	0.039	0.040
<i>Urochroa bougueri</i>	0.002	1.059	0.371	0.020
<i>Urosticte benjamini</i>	0.025	7.872	<0.001	0.074
<i>Urosticte ruficrissa</i>	0.016	3.670	0.020	0.060

CHAPTER 6. GENERAL CONCLUSIONS

Understanding morphological and species diversity has been a major challenge to evolutionary biologists, and for well over a century, examining such patterns has been a major component of macroevolutionary studies. Even before Darwin, in 1753 Linnaeus was beginning to classify and learn about the observable patterns of biological diversity. Later, an explanatory evolutionary theory came into focus with Darwin's two key contributions: natural selection and descent with modification, providing both a natural mechanism for evolutionary change and a description of the resulting patterns through time (Darwin, 1859). Based off of these ideas, we can now begin quantifying and better comprehending the patterns and processes of biodiversity. However, to fully grasp mechanisms driving the evolution of diversity requires that we examine both micro- and macroevolutionary patterns. Indeed, “there is no way toward an understanding of the mechanisms of macroevolution... other than through a full comprehension of microevolutionary processes” (Dobzhansky, 1937). My dissertation examines not only microevolution, but also macroevolutionary patterns and processes, thereby providing hierarchical data to greatly enhance the understanding of evolutionary diversification.

Chapter summaries

In Chapter 2 (published as Berns, 2013) I reviewed, outlined and described several hypotheses and mechanisms that explain patterns of sexual dimorphism within and among hummingbird taxa. I then performed a literature review of studies that focus

on what is known about biological patterns and underlying processes specifically regarding sexual shape dimorphism, the methods used to quantify this and the patterns that arise from these studies. While there are numerous studies that focus on the patterns and processes of sexual dimorphism, many questions remain. For instance, what role does sexual shape dimorphism play in microevolutionary patterns and what are the mechanisms underlying these patterns? What might result when scaling these patterns from micro to macroevolution? Questions such as these could be tested by examining patterns of dimorphism in two closely related species, then scaling up to family, genera and so forth. It is now also possible to ask if rates of evolution differ between species and if these rates differ more broadly between different sexually dimorphic traits. What effect does habitat and environmental gradients play in assessing rates and patterns of sexual shape dimorphism evolution? By examining the possible correlation between sexual shape dimorphism and habitat variables in a phylogenetic manner, it is possible to quantify hypotheses such as these. This chapter served not only as a literature summary, but also pointed to gaps in our knowledge and suggested other studies that might help further our understanding of sexual dimorphism and biodiversity.

In Chapter 3, (published as Berns and Adams, 2010) I examined sexual dimorphism at a microevolutionary level, examining the sexual size and shape dimorphism in the bill morphology of the sister species *Archilochus alexandri* (Black-chinned Hummingbird) and *Archilochus colubris* (Ruby-throated Hummingbird) in the *Mellisugini* hummingbird clade using 4 different techniques to measure bill shape.

From the shape data obtained using geometric morphometric methods, I found that *A. alexandri* does not exhibit sexual shape dimorphism, whereas *A. colubris* does have significant shape dimorphism in its bill morphology. Interestingly, in *A. colubris* the female had the more curved bill as compared to the male, a pattern commonly found in hummingbirds living year-round in tropical regions. Why might two closely related species show this difference? One possibility is that this phenotypic difference may have resulted from the strength of sexual selection differing between species (Andersson, 1994; Møller, 1988; Owens and Hartley, 1998). Another possibility is that differing community structures found in each species' primary habitats may play a role in the evolution of sexual size and shape dimorphism in these two species where, in species-poor communities (such as where *A. colubris* breeds), members of each sex can inhabit ecological niches that would be occupied in species-rich communities (such as where *A. alexandri* breeds; Butler et al., 2007). The lack of potential competitors in the breeding range of *A. colubris* may allow expansion and partitioning of its ecological niche between the sexes by increasing intraspecific competition (e.g., Butler et al., 2007; Dayan and Simberloff, 1994). Examining these species at a microevolutionary level shows an interesting pattern, where one species exhibits sexual shape dimorphism while the sister species does not. This finding is interesting as it is commonly assumed that hummingbirds display sexual dimorphism. It also begs an important question: to what extent is sexual dimorphism displayed across a broader phylogenetic sampling of hummingbirds?

Chapter 4 (Berns and Adams, 2013 in press) scales studies of sexual dimorphism to a clade level perspective by examining patterns across the entire *Mellisugini* clade of hummingbirds. In this study, I found that the majority of species in this clade do exhibit female-biased (females are larger than males) sexual size dimorphism in their bills. This pattern is similar to the microevolutionary finding that both *A. colubris* and *A. alexandri* have significant sexual size dimorphism in their bills. I then compared species in *Mellisugini* to 184 other species of hummingbirds in different clades to find that the presence of size dimorphism was found in majority of species, which was concordant with my prediction based on previous studies, that nearly all species of hummingbirds have sexual size dimorphism in their bill morphology.

I also examined the presence of shape dimorphism in the *Mellisugini* clade, predicting that, as other studies have found on other species, the majority of species would exhibit shape dimorphism. Interestingly however, shape dimorphism was nearly absent in *Mellisugini*, where only 3 of the 32 examined species have shape dimorphism. This lack of shape dimorphism is unusual, particularly in species that reside in the tropics, such as *Selasphorus scintilla* and *Mellisuga minima* (Temeles, 1996; Temeles and Kress, 2003; Temeles et al., 2010; Temeles et al., 2000).

When patterns of sexual dimorphism are examined in light of community composition an interesting pattern emerges, which I suggested to be a factor in the lack of shape dimorphism in *Mellisugini*. Specifically, species generally inhabiting species-poor communities tend to have a greater degree of sexual dimorphism than those living in species-rich communities. This pattern suggests the hypothesis that community

dynamics, perhaps through interspecific competition, may be related to within-species patterns of sexual dimorphism. Under this hypothesis, ecological specialization drives morphological change, and in species rich communities morphological differences thus tend to evolve between species that specialize on distinct resources. By contrast, in species poor communities, males and females within a species can specialize on distinct resources and otherwise unoccupied niches (as there are fewer competing species), thereby facilitating the evolution of greater sexual dimorphism. Taken together Chapters 3 and 4 suggest that sexual size dimorphism is nearly ubiquitous across hummingbirds and that sexual shape dimorphism is rare. Further, these results suggest the hypothesis that community structure and niche segregation may be important factors that underlie the evolution of sexual size and shape dimorphism.

Finally, in Chapter 5 I took a macroevolutionary approach by quantifying sexual size and shape dimorphism in 269 of 331 currently recognized species of hummingbirds (Birdlife.org, 2012; McGuire et al., 2007; McGuire et al., 2009). I used a recent time-dated molecular phylogeny to examine rates of phenotypic evolution in hummingbirds. Specifically, I quantified relationships between rates of evolution of sexual dimorphism, clade age, percent of species with bill dimorphism, species richness, magnitude of dimorphism, and species diversification. Additionally, I tested the hypothesis that at a macroevolutionary scale, patterns of sexual size and shape dimorphism are correlated. Surprisingly, I found that only about half of the hummingbird species examined exhibit significant size dimorphism. This finding is unexpected, as hummingbirds are generally considered to be sexually dimorphic in their bills (Temeles et al., 2010). This finding

also demonstrates the importance of looking at both micro and macroevolutionary scales, as patterns observed within one clade (e.g., the Phaethornithinae hummingbirds: Temeles et al., 2010), may not be present in other clades (e.g., the Mellisugini hummingbirds: Berns and Adams, 2013). Thus, to best understand macroevolutionary patterns, an examination of all clades is preferable to extrapolation from a single representative lineage.

Further, I found that size and shape dimorphism do not associate with one another at a macroevolutionary level such that there was no significant correlation between the mean magnitude of size dimorphism and the magnitude of shape dimorphism. Additionally, the rates of evolution of sexual size dimorphism did not predict those of shape dimorphism and that these traits are not associated suggests that they are evolutionarily decoupled. One possibility to explain this pattern is that different responses of the sexes to environmental gradients may be acting on shape alone, which may drive patterns of shape dimorphism while not affecting size. Another is that the sexes have differential responses in shape, but not size, to predation (Hendry et al., 2006). Future research is needed to assess these evolutionary alternatives.

Intriguingly, the rate of sexual shape dimorphism, species richness, and rate of species diversification are all positively correlated, implying that these are predictors of species richness. These relationships are consistent with the macroevolutionary theory of ‘punctuated equilibrium’ (Gould and Eldredge, 1977), as I found that increased diversification rates are positively related to increase in rate of evolution of shape dimorphism. From this, I hypothesize that patterns in morphology may be due to

differences in speciation and extinction rates, though it is also possible that these patterns result from the opening of new niches and the presence of ‘adaptive zones’ (Simpson, 1944, 1953).

Understanding biodiversity requires that we examine both micro- and macroevolutionary patterns. Microevolution alone cannot explain macroevolution, but instead requires the integration of processes at multiple hierarchical scales; first examining what occurs within species or between closely related species (microevolution), and, once these processes are explored, identifying the processes underlying evolution above the species level (macroevolution). Indeed, in each chapter and at both the micro and macroevolutionary scale, we learn more about the diversity of hummingbirds. My species and clade level studies showed specific ecological patterns and forces impacting the evolution of sexual size and shape dimorphism in the bills of hummingbirds. Without scaling up however, it would remain unknown that patterns of sexual dimorphism in hummingbirds are consistent with punctuated equilibrium and with the idea of adaptive zones. Not only does my research shed light into the evolution of hummingbird diversity, but it also highlights the importance of coupling small and large-scale research to provide insights at numerous levels. In taking this hierarchical approach, I have provided a more complete understanding of biodiversity at several levels and in doing so, ultimately address the longstanding question: “why are there so many species, and why do we see such magnificent biological diversity?”



Figure 1. Hummingbird diversity

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